A STUDY OF CARDIOVASCULAR AUTONOMIC FUNCTIONS AND SERUM LEPTIN LEVELS IN YOUNG OBESE INDIVIDUALS

Dissertation submitted to

The Tamil Nadu Dr. MGR Medical University

In partial fulfillment of the regulations

For the award of the degree of

M.D. PHYSIOLOGY

Branch V

INSTITUTE OF PHYSIOLOGY & EXPERIMENTAL MEDICINE

Government Madras Medical College and Hospital

CHENNAI –600003

THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY

CHENNAI –600032

APRIL, 2016

CERTIFICATE

This is to certify that the dissertation entitled "**A STUDY OF CARDIOVASCULAR AUTONOMIC FUNCTIONS AND SERUM LEPTIN LEVELS IN YOUNG OBESE INDIVIDUALS"** by the candidate **Dr.V.SUMATHI**, for M.D. Physiology is a bonafide record of the research done by her during the period of study (2013 –2016) in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai –600003.

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ACKNOWLEDGEMENT

I express my profound gratitude to **Dr. R. VIMALA, M.D,** the Dean of Government Madras Medical College and Hospital, Chennai, for permitting me to do this study and use all the needed resources for this dissertation work.

I sincerely express my grateful thanks to **Dr.K.PADMA, M.D.,** Professor and Director of Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai, without whom it would have been totally impossible to accomplish this work. I thank her for the thoughtful discussions and providing valuable study materials and for being a constant source of inspiration.

I sincerely express my grateful thanks to **Dr. SRIPRIYA HARIDOSS, M.D,** Assistant Professor, Department of Endocrinology, Madras Medical College and RGGH, Chennai, for providing me with needed subjects and guiding me in this study.

I sincerely express my grateful thanks to **Dr. MINI JACOB, M.D.,** Professor and Head of Department, and **Mrs. ANITHA,** M.Sc. PhD., Central Research Institute, TN Dr. M.G.R. Medical University, Guindy, for helping me to do the lab tests in their department.

I extend my thanks to **Dr. R.VIJAYALAKSHMI, M.D.,** for her valuable guidance rendered throughout my study.

I extend my thanks to **Dr. P.SATHYA, M.D.,** for her valuable suggestions rendered throughout my study.

I extend my thanks to **Dr. A.PARIMALA, M.D** Associate Professors, Institute of Physiology Madras Medical College, Chennai, for her motivation rendered throughout my study.

It is with deep sense of gratitude that I acknowledge my profound indebtedness to Associate Professor, **Dr. C.THIRUPATHI, M.D., D.C.H.,** Institute of Physiology, Madras Medical College, Chennai, for his support and advice rendered throughout my study.

I express my sincere thanks to **Dr. J.RATNA MANJUSHREE, M.D., Dr.KANMANI KARTHIKEYAN, M.D., Dr. JANET SUGANTHA, M.D.,** Assistant Professors, Institute of Physiology Madras Medical College, Chennai, for their constant guidance, motivation, advice and encouragement which enabled me to complete this work.

I express my sincere thanks to **DR. K.AANANDHA SUBRAMANIUM M.D., DR.T.N.VIJAYALAKSHMI, M.D., DR. SHANTHI MALAR, M.D., DR. KAVITHA, M.D.,** Assistant Professors, Institute of Physiology Madras Medical College, Chennai, for their guidance and motivation.

I express my sincere thanks to all the Co Post Graduates in Department of Physiology, Madras Medical College, Chennai.

I sincerely thank and dedicate this work to my lovable family.

I thank GOD ALMIGHTY for helping me throughout this endeavor.

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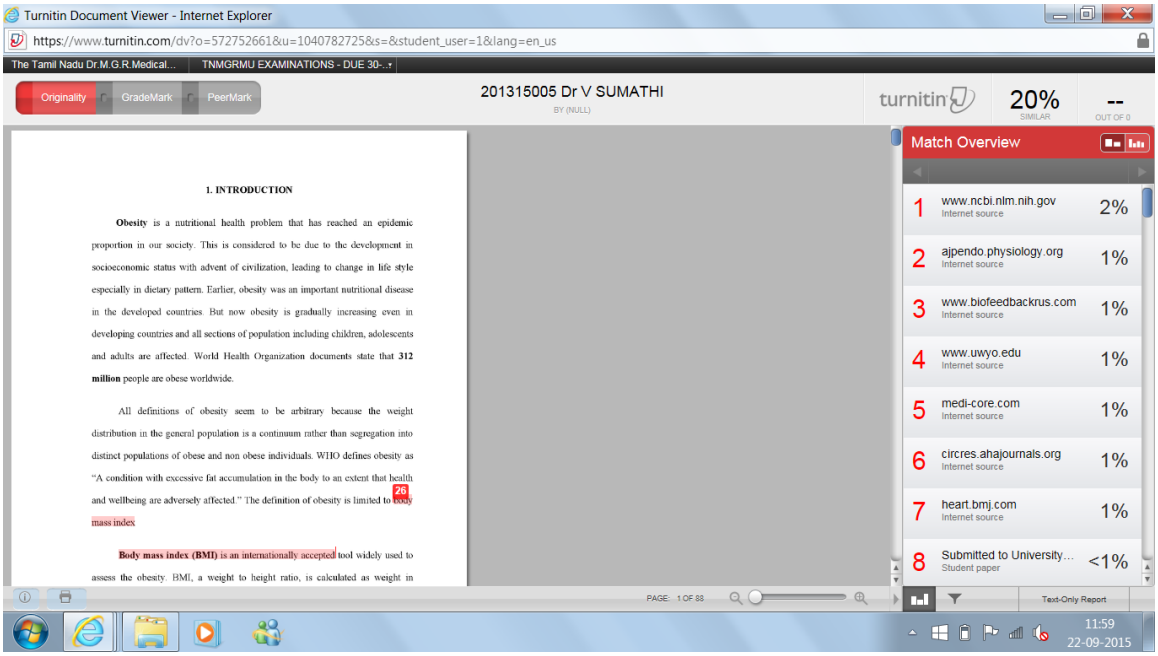
ABBREVIATIONS

ANS	AUTONOMIC NERVOUS SYSTEM
BMI	BODY MASS INDEX
BP	BLOOD PRESSURE
CPT	COLD PRESSOR TEST
DB	DEEP BREATHING
DBP	DIASTOLIC BLOOD PRESSURE
E/I	EXPIRATION/INSPIRATION
ECG	ELECTRO CARDIO GRAM
ELISA	ENZYME LINKED IMMUNO SORBENT ASSAY
FFT	FAST FOURIER TRANSFORM
HF	HIGH FREQUENCY
HR	HEART RATE
IHG	ISOMETRIC HAND GRIP
LF	LOW FREQUENCY
PNS	PARASYMPATHETIC NERVOUS SYSTEM
PNS	PARASYMPATHETIC NERVOUS SYSTEM
SBP	SYSTOLIC BLOOD PRESSURE
SD	STANDARD DEVIATION
SNS	SYMPATHETIC NERVOUS SYSTEM
TMB	TETRA METHYL BENZIDINE
VLF	VERY LOW FREQUENCY
VR	VALSALVA RATIO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1. INTRODUCTION
Obesity is a nutritional health problem that has reached an epidemic proportion in our society. This is considered to be due to the development in socioeconomic status with advent of civilization, leading to change in life style especially in dietary pattern. Earlier, obesity was an important nutritional disease in the developed countries. But now obesity is gradually increasing even in developing countries and all sections of population including children, adolescents and adults are affected. World Health Organization documents state that 312 million people are obese worldwide.
All definitions of obesity seem to be arbitrary because the weight distribution in the general population is a continuum rather than segregation into distinct populations of obese and non obese individuals. WHO defines obesity as "A condition with excessive fat accumulation in the body to an extent that health and wellbeing are adversely affected." The definition of obesity is limited to body mass index
Body mass index (BMI) is an internationally accepted tool widely used to assess the obesity. BMI, a weight to height ratio, is calculated as weight in
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ABSTRACT

A STUDY OF CARDIOVASCULAR AUTONOMIC FUNCTIONS AND SERUM LEPTIN LEVELS IN YOUNG OBESE INDIVIDUALS

Degree for which submitted : Doctor of Medicine(MD) in Physiology

Supervisor and guide : Prof.Dr.K.Padma,
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Department : Institute of Physiology and Experimental Medicine

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Year : 2014 – 2015

Back ground:

Over 312 million people are obese worldwide. Obesity has become a major nutritional health problem in India. It is associated with adverse cardiac events. Obesity is considered as a leading preventable cause of death. Autonomic function tests are early non invasive tool to assess the cardiac distress. Adipose tissue acts as highly active metabolic and secretary endocrine organ. Leptin is one of the main adipocytokines secreted by the adipocytes.

Aim & Objective:

To study the cardiovascular autonomic functions and serum leptin levels in young obese individuals and to compare and correlate with normal individuals

Materials & Method:

This case control study included 50 young obese subjects with BMI ≥ 25 kg /m^2 and 50 controls with BMI 18.5 - 24.9 kg/m^2 of age 18 to 25years. 25 males and 25 females were included in the obese and control group each. After getting an informed consent, the weight, height, body mass index, waist circumference, hip circumference and waist hip ratio were measured. They were made to undergo Resting HRV using Niviqure Ambulatory ECG Recorder, Orthostatic standing test, Deep Breathing test, Valsalva maneuver, Isometric Hand Grip test and Cold Pressor test. 5 ml of venous blood was collected for estimating serum leptin levels by ELISA method. The results obtained were analyzed with SPSS version 21 and compared with normal individuals using independent student t test.

Result:

The weight, body mass index, waist circumference, hip circumference and waist to hip ratio were all increased significantly in the young obese individuals. The resting systolic and diastolic blood pressure in obese group though within normal limits was significantly increased. In Resting HRV, the time domain measures SDNN was reduced, Mean HR was increased significantly in the obese group. The frequency domain measures LF nu and LF/HF ratio were increased and HF nu was reduced significantly in the young obese individuals.

In Orthostatic standing test 30/15 ratio was decreased, and the variation in SBP and DBP at 1 minute on standing was significantly more in the obese group.

E/I ratio in deep breathing test, and Valsalva ratio were reduced significantly in the obese group. There was a significant increase in the variation of SBP and DBP in the Isometric test and DBP in Cold pressor test in the obese group. In young obese individuals, Serum leptin levels are significantly higher correlating with BMI and waist circumference and with HRV parameters.

All these results substantiate autonomic dysfunction with sympathetic predominance and parasympathetic withdrawal.

Conclusion:

Autonomic Function tests in young obese individuals indicate that there is a definite sympathovagal imbalance in the form of sympathetic over activity and parasympathetic withdrawal. Elevated serum leptin levels play a direct vital role in stimulating the sympathetic nervous system. Chronic activation of the sympathetic nervous system makes them more prone for adverse cardiovascular events.

The battery of autonomic function tests is an effective tool in identifying the autonomic dysfunction at an earlier stage. Understanding the role of the sympathetic nervous system and the serum leptin levels in obesity might help in the treatment of obesity and prevent further complications in this disease. Early diagnosis, behavioral and life style modification, health education and medication, may alleviate such problems.

Keywords: Young obese, Resting HRV, cardiovascular autonomic functions, Serum Leptin, sympathovagal balance.

1. INTRODUCTION

Obesity is a nutritional health problem that has reached an epidemic proportion in our society. This is considered to be due to the development in socioeconomic status with advent of civilization, leading to change in life style especially in dietary pattern[1]. Earlier, obesity was an important nutritional disease in the developed countries. But now obesity is gradually increasing even in developing countries and all sections of population including children, adolescents and adults are affected[2]. World Health Organization documents state that **312 million** people are obese worldwide.

All definitions of obesity seem to be arbitrary because the weight distribution in the general population is a continuum rather than segregation into distinct populations of obese and non obese individuals. WHO defines obesity as "A condition with excessive fat accumulation in the body to an extent that health and wellbeing are adversely affected." The definition of obesity is limited to body mass index.

Body mass index (BMI) is an internationally accepted tool widely used to assess the obesity and it is an internationally accepted tool. BMI, a weight to height ratio, is calculated as weight in kilograms by height in meter square[3].

Present environment of excessive food availability and intake as compared to decreased physical activity favors an increase in adiposity. Thus an excessive

intake of calories in relation to energy expenditure which is termed as **positive energy balance**, over a long period of time leads on to obesity.

Obesity was rare before the 20th century (Haslam et al, 2007). The WHO formally recognized "obesity as a **global epidemic** in 1997" (Caballero, 2007). At least 9.8% of the general population are obese, with higher occurence among women than men according to WHO in 2005. Obesity growing as an epidemic in many countries (Friedman and Fanning, 2004) has become a major public health problem (Friedrich, 2002), affecting a wide spectrum of age groups (Pischon et al, 2008), from young to elderly (Bellows-Rieckel et al, 2008). Among children and adults the estimated prevalence ranges from 10 to 50 percent (Van Gaal et al, 1995). The current International Obesity Task Force estimates suggest that, at least 1.1 billion people are overweight worldwide and out of which 312 million of them are obese.

Obesity is a **pro inflammatory condition** that increases cardiovascular morbidity and mortality risk by various mechanisms[4,5,6].Obesity is associated with alterations both in hemodynamics and metabolic activity. Obese people have higher prevalence of **co-morbidities** like hypertension, hyperlipidemia, coronary heart disease, Type 2 diabetes mellitus, Sleep Apnea Syndrome, osteoarthritis, fatty liver, polycystic ovarian disease and neoplasms. These co-morbid conditions are the cause of increased rate of death in obesity. Obesity forms a part of the metabolic syndrome, which is diagnosed by the presence of central obesity,

dyslipidemia, hypertension, insulin resistance who have a higher risk for type 2 diabetes and cardiovascular diseases [7]. Early studies indicate that obesity is associated with **sudden cardiac deaths** [8,9]. Hence obesity is considered as a leading preventable cause of death worldwide (Ezekiel et al, 2008).

Several approaches, which include direct techniques and indirect techniques have been taken to measure obesity. The **direct techniques** involve a number of procedures that include densitometry, measurement of total body potassium, estimation of total body water, neutron activation techniques, computed topography (CT scan), magnetic resonance imaging scans (MRI scans), electrical methods that measure conductivity and impedance.

The **indirect techniques** for estimating total fat include skin fold measurements, weight to height ratios, waist circumference, waist to hip ratio and hip circumference.

"**Body weight** above 20% of the ideal weight is obesity". Height, age, sex and built of an individual are taken into account for ideal body weight.

Waist Circumference is measured in centimeters at a level of umbilicus or midway between lower rib cage and pubic symphysis. It is also a good indicator of total body fat. It is the best anthropometric indicator of visceral fat. WC $\geq$ 102 cm in males and $\geq$ 88 cm in females indicates obesity.

"**Waist Hip Ratio (WHR)** is the ratio of waist circumference to hip circumference." The hip circumference is measured as widest circumference at the level of greater trochanter. Though difficult to measure, WHR is a useful predictor of risk of cardiovascular disease and diabetes in adults. WHR ≥ 1.0 for males and WHR ≥ 0.9 for females indicates obesity.

Skin fold thickness measures the subcutaneous fat that correlates with total body fat. Using skin calipers skin fold thickness is measured in mid triceps, biceps, supra iliac and supra scapular regions. Sum of these measurements more than 40 mm in males and more than 50 mm in females indicates obesity.

In 1997, it was proposed that thresholds shown in the below table shall be used to classify overweight in adults according to BMI (WHO, 1998)

BMI	**Classification**
< 18.50	**Underweight**
18.50 – 24.99	**Normal Range**
≥ 25.00	**Overweight**
25.00 - 29.99	Pre obese
30.00 - 34.99	Class I Obese
35 - 39.99	Class II Obese
≥ 40.00	Class III Obese

"World Health Organization defines obesity as a BMI of 30 kg/m^2 or greater, and overweight as a BMI between 25 kg/m^2 and 29.9 kg/m^2"(WHO,2002)

The Asian populations develop health consequences at a lower BMI, so it has been proposed that a BMI of 25 kg/m^2 or greater should be classified as obesity (James et al, 2001).

"**Body fat percentage** is the total weight of body fat divided by body weight". A body fat percentage ≥ 23% in males and ≥ 29% in females indicates obesity. Body Fat Percentage (BF %) can be calculated from BMI in adults using the equation given below.

BF% = (0.23 x Age) + (1.2 x BMI) - (10.8 x Sex) - 5.4

Where, BMI is in Kg/m^2; Sex=0 for female and 1 for male; Age is in yrs;

Large scale epidemiologic studies suggest that cardiovascular and metabolic morbidities begin to rise when BMI is ≥ 25. The relative risks of death due to coronary disease for the increasing levels of BMI are given below showing that there is a proportionate increase in risk with the degree of obesity.

Relative risk	BMI
1	< 19
1	19 – 21.9
1.4	22 – 24.9
1.7	25 – 26.9
3.1	27 - 28.9
4.6	29 - 31.9
5.8	>32

The Autonomic Nervous System (ANS) through its two divisions sympathetic and parasympathetic systems work in a coordinated manner either acting reciprocally or synergistically and regulate visceral functions. Autonomic imbalance with reduced vagal tone and increased sympathetic activity has been implicated in pathophysiology of arrhythmias and adverse cardiac events[10,11,12].

A human heart in a healthy state does not beat at a regular rate. There occurs a variation from one beat to the next beat. Even under resting condition, the duration of cardiac cycle of all heart beats is not the same. This spontaneous variation is known as the Heart Rate Variability (HRV).

Thus, Donald Moss and Fred Shaffer aptly described that "The human heart is a bio-electrical pump, beating at an ever changing rate; it is not like a clock that beats at a steady unchanging rate".

The heart rate is regulated by the autonomic nervous system and it is under the constant influence of external and internal stimuli. Metabolic and cardiovascular disorders are associated with autonomic dysfunction, leading to compromise in blood pressure and heart rate thus resulting in cardiovascular mortality.

In un-innervated human heart, the intrinsic firing rate of Sino Atrial Node (SA node) is 100 beats per minute (**WJ Ganong**) whereas in an innervated heart, the parasympathetic innervation supplied by vagus nerve, decreases the firing rate of SA node while the sympathetic system will increase the rate of depolarization of SA node. The net effect of the accelerating influences of the cardiac

sympathetic system and the decelerating influences of parasympathetic system will determine the cardiac sympathetic tone and parasympathetic tone correspondingly. The predominance of resting vagal tone over sympathetic tone is responsible for heart rate below 100.The heart rate (HR) is conventionally measured by counting the number of heart beats per minute.

R-R interval is the time interval between successive heartbeats which can be obtained from electrocardiogram (ECG) or plethysmogram. The sympathetic activity will decrease the RR interval and parasympathetic will increase the RR interval.

The evaluation of autonomic disorders is available more widely with the advent of more reliable, non invasive techniques. The most commonly used autonomic function tests relies on heart rate and blood pressure and the changes in response to breathing and to posture variations. These tests are simple, non invasive tests, easy to perform and reproduce, and are both sensitive as well as specific.

Greater heart rate variability implies that the heart adapts more quickly and flexibly to internal and external influences due to an optimum interplay between the PNS and SNS. Lower heart rate variability indicates a reduced ability for adaptation which in turn may suggest autonomic dysfunction leading to serious health impairment.

HRV is considered as an early tool that reflects distress. It is an early warning sign of alteration in homeostasis to evaluate the integrity and functional state of ANS.

The fact that autonomic innervation of SA node is responsible for variability of heart rate has been utilized to develop tests that stimulate this autonomic supply and produce variability of heart rate. These tests evaluate the cardiac division of the ANS, both sympathetic and parasympathetic system. They are grouped as cardiac autonomic functions tests. They test the autonomic reflex pathway, where the heart is the effectors end organ. The autonomic reflexes have an afferent pathway, a center that help in processing and integrating and an efferent pathway like other reflexes. These tests assess the resting heart rate variability and its variability in response to a challenge.

Task Force (1996) has designed and recommended a battery of tests to evaluate the autonomic functions[13]. The cardiovascular autonomic function tests includes orthostatic standing test, deep breathing test, isometric handgrip test, cold pressor test and resting heart rate variability.

Leptin was identified in 1994 by J.M.Friedman. Leptin is a 16 kDa protein, consisting of 167 amino acids [14] . It is produced by adipocytes under the neuroendocrine control [15] . In humans, the "Ob"(Lep) gene is located on chromosome 7. Leptin functions as an anti-obesity hormone primarily.

By increasing overall sympathetic nerve activity, leptin reduces appetite, while it enhances energy expenditure [16]. It improves insulin sensitivity and facilitates glucose utilization. The circulating leptin level is considered to be in direct proportion to the total amount of body fat[17]. Insulin sensitivity may be affected by leptin either through direct peripheral effects in insulin-sensitive tissues or by centrally mediated mechanisms. Thus, leptin may regulate glucose metabolism with hypothalamus as center via central and/or peripheral pathways[18].

As such elevated plasma leptin levels may be an independent risk factor for the development of cardiovascular disease. Leptin that is considered a satiety factor thus increases energy expenditure by activating the sympathetic nervous system. There are also evidences which state that chronic infusion of leptin increases heart rate and blood pressure.

Therefore in this present study, the autonomic activity of young obese individuals are assessed by a battery of autonomic function tests, where obesity is a pro-inflammatory condition and also their serum leptin levels, which is a adipocytokine are assessed and compared and correlated with that of controls of normal BMI.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Obesity has been known since human existence. Earlier it was considered as a symbol of prosperity. In ancient days, there was scarcity of food and adipose tissue remained as reserve of energy for utilization during the period of starvation. Some of the milestones in obesity are:

- In ***Old Stone Age***, obesity was depicted in many artefacts, of which '***Venus of Willendorf****'* found in Austria, showing a marked abdominal obesity and pendulous breast was the most famous one.
- In ***New Stone Age***, with the advent of agriculture and food security, many artefacts including 'Mother Goddess' found in Turkey depict obesity.
- ***Hippocrates***, a Greek physician said that "Corpulence is not only a disease itself, but the harbinger of others. Sudden death is more common in those who are naturally fat than in the lean." Obesity as a cause of infrequent menstruation and infertility in women was also noted by Greek physicians.
- ***Galen***, a Roman physician distinguished between moderate and immoderate (morbid) forms of obesity.

"Obesity is characterized by excessive accumulation of fat in the body to an extent that it may have an adverse effect on health, leading to decreased expectancy of life and/or increased health problems" (WHO,2000) [19] . The definition of obesity is limited to BMI and to increases in adipocyte cell size and/or cell number (Bray GA et al, 1998)[20]. In 1985, in a Consensus Development

Conference on Health Implications of Obesity held by The National Institute of Health, obesity was concluded to be a disease.[21]

Causes of Obesity:

The common causes of obesity are positive energy balance and genetic susceptibility while a few cases are due to endocrine disorders, psychiatric illness, or medications. Modernization of lifestyle with physical inactivity, increased stress and easy availability of high caloric diet has probably lead to current level of epidemic of obesity. (James et al)[22]

Classification of obesity:

Obesity has been classified based on variety of features

a) ***Anatomical distribution of fat:*** Peripheral obesity (pear shaped, gynoid, gluteo-femoral or lower body obesity) and Central obesity (apple shaped, android, abdominal or upper body obesity)
b) ***Morphology of adipose tissue:*** Hyperplastic obesity (increased number of fat cells) and Hypertrophic obesity (enlarged fat cells)
c) ***Period of onset:*** Juvenile, Mature or pregnancy related.

Adipose tissue:

Adipose tissue is of two type namely white or yellow adipose tissue, which forms the major part in human adipose tissue and brown adipose tissue (BAT)

which is present in significant amount in neonates to help in temperature regulation.

Functions of adipose tissue:

Energy storage and release of fatty acids when fuel is required was the traditional role attributed to white adipose tissue (Henry CJK et al, 1990)[23]. Adipose tissue is considered the body's largest energy reservoir (Collins S et al, 1995)[24]. It is basically required for glucose homeostasis to be normal.

Brown adipose tissue (BAT) is concerned with adaptive thermogenesis. In contrast to white adipose tissue, BAT expends stored energy as heat which it accomplishes through a unique mitochondrial protein namely thermogenin or uncoupling protein (UCP- 1) that uncouples fuel oxidation from ATP generation by breaking the hydrogen ion gradient across inner mitochondrial membrane.

The metabolic role of white adipose tissue is complex and this tissue has multiple functions. It also acts as an endocrine organ. Siiteri PK et al in 1987 first suggested that adipose tissue may have endocrine function by identifying its ability to interconvert steroid hormones[25].

Adipose tissue normally acts as a whole as a single functional unit. It is identified that adipose tissue in different parts of the body may vary in their functions, the upper body subcutaneous tissue releasing major amount of systemic non esterified fatty acids (Martin ML et al, 1991)[26] and also their proximity to the lymph nodes play a role (Pond CM et al, 1995)[27].

Though the main function of adipose tissue is energy storage, other functions of synthesis and secretion of proteins that regulate various body functions are listed below.

PROTEINS SECRETED BY ADIPOSE TISSUE		**ROLE**
Leptin Adenosine Acylation stimulating protein	Friedman,Halaas et al[28] Kather et al[29] Sniderman et al[30]	Regulation of fuel flux
Adiponectin TNF-α	Weyer et al[31] Moller et al[32]	Regulation of insulin action
Angiotensin converting enzyme Angiotensinogen PGI2	Gorzelniak et al[33] Van Harmelen et al[34] Fink et al[35]	Regulation of vasomotor tone
TNF-β PGI2 IGF-1	Alessi et al[36] Negrel et al[37] Wabitsch et al[38]	Regulation of cell turn over
Interleukin – 6 Adipsin TNF – α	Hirano et al[39] Esterbauer et al[40] McDermott et al[41]	Regulation of inflammation
17 β – OH steroid Cytochrome P 450 dependent aromatase	Crobould et al[42] Bulun et al[43]	Steroid conversion, reproduction, bone mass
Plasminogen activator inhibitor-1 PGI2	Crandall et al[44] McCarty et al[45]	Regulation of coagulation
Agouti signal protein	Voisey et al[46]	Others

Adipocytokines:

Adipose tissues produce a wide range of proteins termed Adipocytokines. Adipocytokines highlight that these proteins may also act locally in an autocrine and paracrine fashion in addition to acting on distant organs in an endocrine fashion. Adipose tissue can thus regulate its own function through adipocytokines.

Regulation of fatty deposits :

Functions of adipose tissue are regulated by various factors such as rate of blood flow, autonomic nervous system activity and the delivery of hormones and substrates in the plasma. The metabolism in adipose tissue is complex and dynamic. The triacylglycerol (TG) stored in adipocytes depicts the net balance between fat deposition and mobilization.

Blood supply of adipose tissue:

It is around 3ml/100gms/min after an overnight fast[47]. The blood flow is highly labile. It increases in response to a meal, prolonged exercise, prolonged fasting.

Expanding fat stores in obesity requires new blood vessels for blood supply. Angiogenesis, a process of new blood vessel formation, occurring in adipose tissue appears to be regulated by factors secreted by the adipose tissue itself like Leptin which has angiogenic properties[48] and matrix metaloproteinases which are involved in vascular remodeling[49].

The adipose tissue releases non esterified fatty acid (NEFA) into the circulation. This transport needs a supply of albumin and thus an increased substrate delivery for clearance of triacylglycerol.

Innervation of adipose tissue:

ANS innervates white adipose by, both sympathetic which control catabolic processes and parasympathetic divisions which control anabolic processes (Bartness T J et al, 1989)[50]. The sympathetic nervous system (SNS) releases neurotransmitter noradrenaline. The catecholamines, adrenaline and NA and the antagonist or agonists of the adrenoceptors can influence the blood flow to the adipose tissue. Alterations in number and subtypes of adrenoceptor occur in obesity (Lafontan M et al, 1995)[51]. Energy stores and adipocyte size modulate the autonomic signals to adipose tissue. Parasympathetic innervation of adipose tissue when stimulated decreases lipolysis (Kreier F et al, 2002)[52].

Fat deposition:

The de novo lipogenesis in human fatty tissue is less (Shrago et al)[53] and insiginificant except when there is overfeeding (Hellerstein et al, 1996)[54]. Insulin stimulates both adipose tissue lipoprotein lipase activity and esterification, leading to dietary TG storage in the postprandial period. Increase in vascularity of adipose tissue during postprandial period may be involved in this TG storage in the adipose tissue.

Steroid hormones like sex steroids and glucocorticoids may regulate the fat storage in specific sites.

Acylation-stimulating protein (ASP), a peptide containing 76 aminoacids, is also found to stimulate fat deposition by stimulating esterification of fatty acid in adipose tissue. The presence of chylomicrons in vitro stimulates the production of acylation stimulating protein.

Fat mobilization:

Activation of hormone-sensitive lipase stimulates fat mobilization pathway. Increased gene transcription may also be involved.

Insulin suppresses the pathway of fat mobilization leading to suppression of release of non esterified fatty acids from subcutaneous adipose tissue. Catecholamines activate lipolysis, via β-adrenergic receptors. Growth hormone and cortisol also modulates fat mobilization. It has been suggested that Atrial natriuretic peptide (ANP) also activates HSL.

Differentiation of adipose tissue:

Preadipocytes can differentiate into mature adipocytes throughout life (Hauner H et al, 1989)[55]. Insulin and fatty acids are important signals for such differentiation. Fatty acids act via PPARγ and PPARδ of the peroxisome proliferator-activated receptor family[56]. Probably a fatty acid derivative is the natural ligand for PPAR (Forman BM et al, 1995)[57].

Insulin regulates adipose tissue differentiation through sterol regulatory element binding protein-1c[58]. Adipocytes in different stages of development may vary in adipocytokine production. The production of TNF-α is less in preadipocytes than in mature cells.

Obesity as an inflammatory disease:

White fat also plays a role in inflammatory processes. Adipsin (Esterbauer et al, 1999), Interleukin – 6 (Hirano et al, 2000), TNF – α (McDermott et al, 2001) secreted by adipocytes play a great role in inflammation and hence obesity is considered a pro-inflammatory condition. Obesity is identified to be a low grade chronic inflammatory disease associated with infiltration of macrophages and expression of high levels of inflammatory markers.

Yamaguchi et al, 2005 has stated that these inflammatory markers contribute to systemic inflammation**.** The number of macrophages infiltrating the adipose tissue has a strong correlation with body mass index, total body fat, body weight (Weisberg et al, 2003).

In obesity the enlarging mass of adipose tissue results in hypoxia well before the occurrence of angiogenesis. This hypoxia leads to recruitment of a transcription factor namely hypoxia-inducible factor-1 (HIF-1) (Trayhurn et al**,** 2004). HIF-1 acts as a chemotactic agent and induces infiltration with inflammatory cell especially macrophages and results in local as well as systemic inflammation.

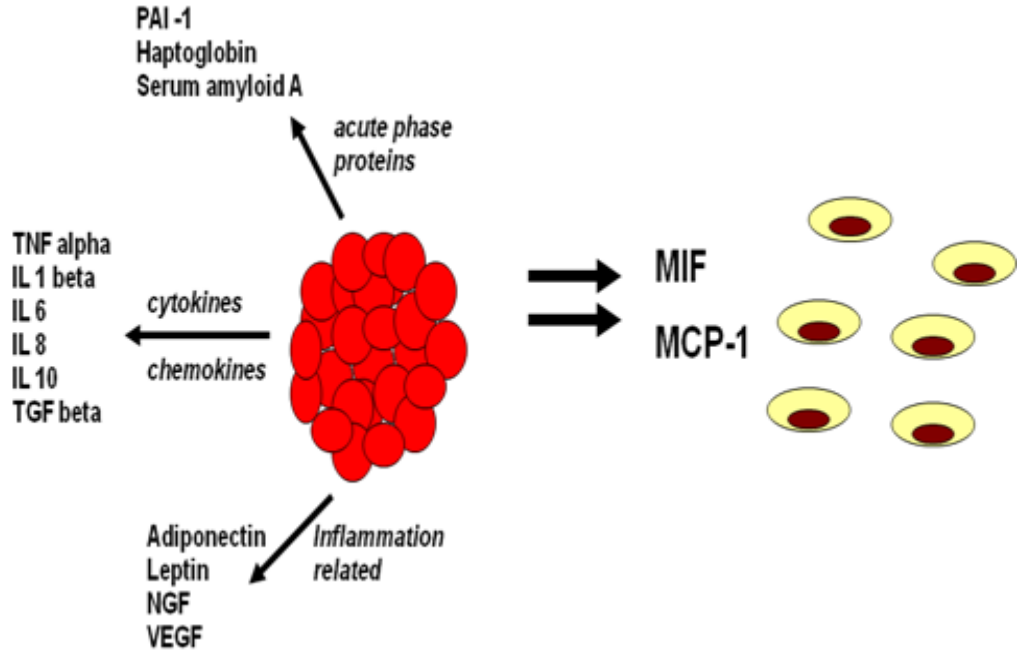


Figure- 1. Pathogenesis of inflammation in obesity

The inflammatory cytokines released from the adipocyte as a result of inflammation namely adipokines are of more than 50 different types like tumour necrosing factor- α (TNF-α), many interleukins and inflammation-related signals such as Monocyte chemo attractant protein-1 (MCP-1), Macrophage migration inhibiting factor (MIF), nerve growth factor, and adiponectin which are different in structure and of function.

Out of these adipokines, the rate of secretion and plasma levels of MIF, its mRNA and MCP-1 positively correlated with BMI (Sartipy and Loskutoff et al, 2003, Skurk et al, 2005). Thus "MIF and MCP-1 may be considered as key obesity-dependent mediator of macrophage infiltration of adipose tissue".

Circulating levels of IL-6, C-reactive protein, TNFα and its soluble receptors, IL-18 and acute phase reactants like plasminogen activator inhibitor-1 and haptoglobin, Amyloid A are also increased in obesity. These evidences support the basis that the obesity is characterized by chronic low grade inflammation in adipose tissue associated with obesity.

Obesity associated inflammation induces oxidative stress:

In obesity, oxidative stress markers derived from oxidation of low-density lipoprotein like lipid peroxides, 8-hydroxydeoxyguanosine and nitrative stress markers like 3-nitrotyrosine and 3-chlorotyrosine are elevated along with high-sensitivity C-reactive protein (hsCRP), which is an early indicator of inflammation (Ignazio et al, 2007). Major source of increased oxidative stress in obesity is NADPH oxidase.

There occurs an imbalance between the production of free radicals particularly high levels of reactive oxygen species and their scavenging mechanism that is low antioxidant enzyme protein content like manganese, copper, zinc super oxide dismutase and catalase which results in disturbances of cellular redox homeostasis (Shattock et al, 1995).

Hence, the excess production of oxygen free radicals in obesity causes injury to deoxyribonucleic acid (DNA), lipid membranes, proteins, and other cellular components. Peter Viitala et al., (2008) reported that the free radical damage to lipid membrane results in lipid peroxidation. “Lipid peroxidation is

defined as the oxidative deterioration of polyunsaturated fats in cellular lipid membranes caused by oxygen free radicals and its propagation".

Net effect of inflammation and oxidative stress on obese individuals:

The oxidative stress, inflammation and nitrative stress increased in obesity contributes to the pathogenesis of Metabolic syndrome which includes diabetes mellitus, hypertension, hyperlipidemia and obesity **(**Yu Yamaguchi et al., 2005) and various above mentioned associated co morbid conditions like aging, Alzheimer's disease, kidney disease, neurodegenerative disorders and cancers (Peter and Keaney et al, 2003).

Obesity- Changes in Cardiovascular functions*:*

A variety of cardiovascular changes from a hyperdynamic circulation to overt heart failure may occur in obesity [59] . Obesity is associated with haemodynamic overload [60] . The increased metabolic demand imposed by the expanded adipose tissue and augmented fat-free mass in obesity results in a hyperdynamic circulation with increased blood volume leading to the increased preload.

In addition left ventricular afterload shows a raise in obesity because of increase in peripheral resistance and arterial stiffness[61]. Right ventricular afterload

may be elevated, possibly due to associated sleep apnea and left ventricular alterations[62].

Cardiac output is raised in obesity, due to raise in stroke volume and heart rate due to increased sympathetic outflow. Intolerence to exercise occurs in obesity, as their ejection fraction do not accentuate with exercise[63]. The increased resting left ventricular end diastolic volume in obesity has been interpreted as evidence of enhanced recruitment of preload reserve based on Frank-Starling mechanism.

Obesity adversely impacts cardiac diastolic function. Elevated systemic oxidative stress and renal sodium retention happening in obesity (Keaney JF et al, 2003)[64] and proinflammatory cytokines from adipocytes (Lyon CJ et al, 2003)[65] may be involved in pathogenesis of left ventricular dysfunction. A straight lipotoxic effect on heart too occurs (Chiu HC et al, 2001)[66].

Atrial and ventricular remodeling, aggravated by hypertension, which are considered to lead to atrial and ventricular dysfunction respectively occur in obesity. Obese individuals have larger left atrium is enlarged, due to increased intravascular volume and changes in LV filling. Obesity is associated with eccentric (being more common) and concentric types of left ventricular hypertrophy (Lauer MS et al, 1992) [67] . Thus obesity is associated with cardiomyopathy.

Autonomic Nervous system (ANS):

The term autonomic nervous system was coined by Langely in 1898 to describe that part of nervous system which controls the automatic, unconscious functions. ANS is an involuntary system and as it controls the vegetative functions it is also called as vegetative system. The autonomic nervous system is responsible for the motor control of the viscera hence called the visceral motor system.

The visceral afferent fibers originate from sensory receptors in the viscera carrying sensations like hunger, thirst, nausea, pain and a sense of visceral distention. Whenever internal stimuli signals about derangement of the internal environment, its autonomic nerves and the central nervous system (CNS) commands compensatory actions. Thus the ANS collects the information about the changes that takes place in the internal environment, interprets these changes and guides the actions and gets the plan executed with the help of effector organs of ANS like smooth muscles of viscera, cardiac muscles and secretory epithelium of glandular tissues. The autonomic nervous system assists the body in maintaining the constancy of internal environment (homeostasis).

Divisions of ANS:

The autonomic nervous system is further divided into sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), each having a central and peripheral component.

The sympathetic preganglionic neurons are situated in the intermedio-lateral horn of thoracic and upper lumbar segments (T1 to L2) of the spinal cord hence the name **thoraco-lumbar division**. The parasympathetic preganglionic neurons are found in the brainstem and in the intermediolateral grey horn of sacral spinal cord (S2 to S4) hence the name **craniosacral division**. Their axons leave the central nervous system and they synapse in specialized ganglia.

Sympathetic postganglionic neurons are located either in paravertebral or prevertebral ganglia. Parasympathetic postganglionic neurons are located in ganglia, which lie close to or within the walls of the target organs. Postganglionic neurons (Second order neurons) innervate smooth muscle and cardiac muscle directly.

Higher centers of ANS:

Higher centers reside in brainstem, limbic system and hypothalamus. Since hypothalamus plays a vital role in the regulation of autonomic activity, it has been called the main ganglion of the ANS. Higher centers have vital connections with the preganglionic neurons to integrate and regulate visceral functions in order to maintain homeostasis.

Most of the viscera have both sympathetic and parasympathetic innervations. The sympathetic and parasympathetic nervous systems of the organs work in a highly coordinated manner, sometimes acting reciprocally and at times

synergistically to regulate visceral functions in order to maintain the internal environment.(Berne and Levy,6th edition)[68]

Neurotransmitters in ANS:

Acetylcholine is the primary neurotransmitter in both pre ganglionic and postganglionic neurons of the parasympathetic system. Nor epinephrine is the primary neurotransmitter in sympathetic end organ targets with two main subtypes of alpha and beta receptors. Otto Loewi in 1920 provided the first evidence for chemical neurotransmission by performing a simple yet dramatic study of heart rate control by the parasympathetic nervous system. (Ganong, 23rd edition)[69]

Cardiovascular autonomic nervous system:

Both sympathetic and parasympathetic nerves innervate the heart and the vascular system. ANS of the heart is under the control of cardiac autonomic centers situated in the medulla, which integrates the cardiac autonomic reflex. Vasomotor center is the primary cardiovascular regulatory center located in the medulla oblongata of brainstem.

The sympathetic supply is controlled by cardiac excitatory area (Pressor Area) in Rostral Ventero Lateral Medulla (RVLM). The cervical sympathetic nerves reach the heart, thus forming the efferent limb of cardiac autonomic reflexes. The parasympathetic center is located in nucleus ambiguous and dorsal motor nucleus of vagus and its output reaches the heart through the vagus nerve.

Afferent inputs from various central and peripheral organs also reach these cardiac autonomic centers. Some of the inputs excite the cardiac sympathetic centers, which in turn inhibit the parasympathetic centre. Thus there is an antagonist effect of these vital centers on each other.

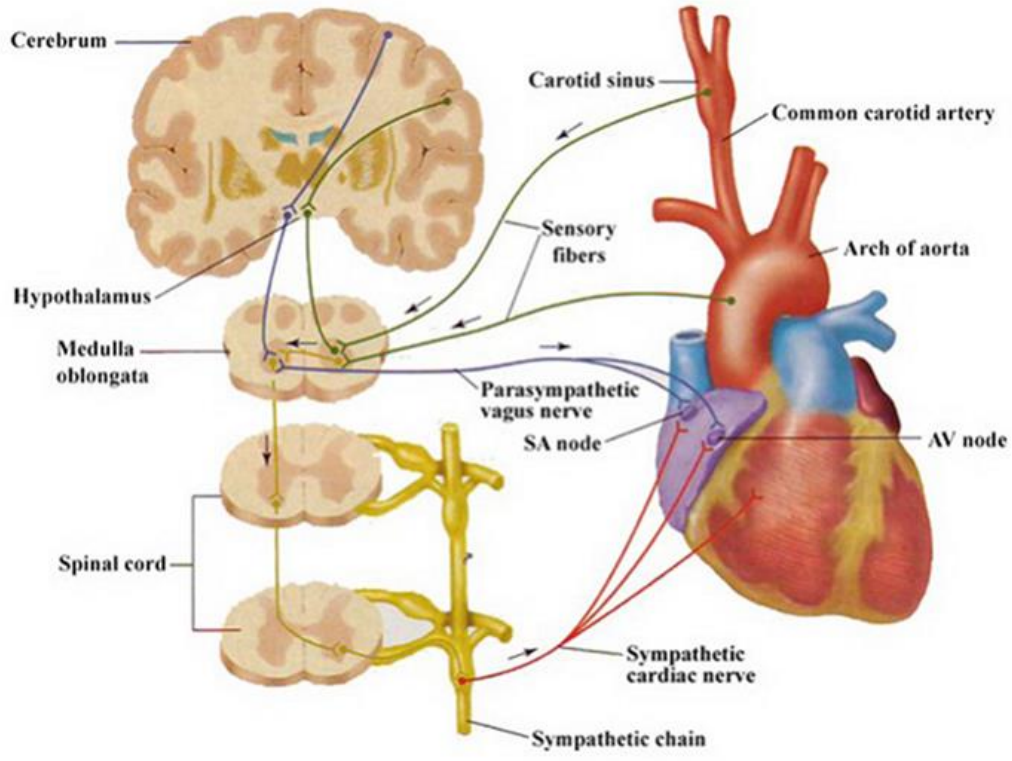


Figure -2. Autonomic innervation of heart.

Heart is innervated by both divisions of autonomic nervous system namely sympathetic and parasympathetic nerves. Sympathetic preganglionic fibers originating in the intermediate horns of upper 4 or 5 thoracic spinal segments relay in stellate ganglion, then reach the heart and innervate the pacemaker, the conducting system, atrial and ventricular muscles. The vagus nerve

(parasympathetic) originates from the dorsal nucleus of vagus in medulla, then ends in ganglia close to sinoatrial and AV node and the post ganglionic nerves which are short and supply the pacemaker, the conducting fibers, and also sparsely innervates the muscle fibers.

The vagus innervates the SA node (the pacemaker) by its right branch, AV node by its left branch and the conducting system of heart. Heart has its own inherent activity which is visualized as spontaneous firing of pacemaker cells situated in the wall of right atrium. These pacemaker cells of SA node are specialized cardiac muscle cells with large number of gap junctions and scanty contractile fibers. Many local factors such as changes in the hormone, temperature and stretch of the SA node change the heart rate. Above all, a vital role is played by ANS in controlling heart rate effectively.

On stimulation, vagus releases acetylcholine from the post ganglionic nerves which makes the cardiac fibers less excitable. The acetylcholine increases the potassium permeability leading to increased potassium efflux resulting in hyper polarization of the membrane, the end result being reduced excitablility of the tissue. Vagal stimulation leads to reduction in the rate of pacemaker impulses, slowing of conduction in the conducting system, reduction in the force of contraction of atrial muscles and the ventricle muscle is least affected and thus vagus is cardio inhibitory.

The impulses from the cardio respiratory region of medulla reach the heart through the vagus and keep a check of heart rate which is called as vagal tone. The

vagal tone originates from the sinoaortic baroreceptors, the impulses then reach medullary cardio inhibitory center and send impulses to heart through vagus. Denervation of the SA node causes abolition of vagal tone.

The sympathetic stimulation is chronotrophic (increases the heart rate), inotropic (increases the force of contraction), dromotropic (increases the conduction velocity) and bathmotrophic (increases the excitability). Sympathetic stimulation releases Noradrenaline from the post ganglionic nerve terminals which causes a reduction in potassium efflux and opens the transient calcium channels in SA node. The opening of L type of calcium channels in the cardiac muscles increase the force of contraction. These actions are mediated by β_1 receptors in the cardiac tissue. (Frielle T et al)[70].

There is some degree of sympathetic tone along with vagal tone and sympathetic denervation reduces the heart rate considerably. Sympathetic tone also maintains the vessels in partially constricted state thus maintaining the vascular resistance and playing a vital role in the maintenance of the blood pressure.

Normally there exists a balance between sympathetic and parasympathetic tone and called as sympathovagal balance. In a denervated heart, where both sympathetic and parasympathetic nerves are blocked, the heart beats at the rate of 100 -110 beats/ minute which is called as the intrinsic heart rate. But the normal adult resting heart rate is around 72 beats / minute which suggest that under resting conditions the parasympathetic tone predominates.

Autonomic Function Testing:

Rather than relying on a single test, it is preferable to perform a battery of tests to determine the intactness of autonomic reflex (Ewing and Clarke et al, 1982)[71]

The cardiovascular autonomic function tests that relies on blood pressure and heart rate and the changes in response to breathing, and to postural variations are frequently used for testing the integrity of autonomic nervous system and thereby the regulation of homeostasis (Genovelyland et al, 1998)[72]. The fact that variability of heart rate occurs because of autonomic innervations of SA node has been exploited to develop tests that stimulate this autonomic supply and produce variability of heart rate.

These tests which are simple, easy to perform and reproduce, non invasive, sensitive as well as specific are listed below:

Non invasive tests of Autonomic Function:

These tests were adapted from Text book of clinical evaluation of diagnostic test for NM disorders. (Annabel K. Wang)

Tests for Sympathetic nervous system:

- Heart rate and BP response to active standing
- Beat to Beat Blood pressure response to Valsalva maneuver

- BP and HR response during and after 5 minutes of isometric hand grip test
- Diastolic BP and heart rate response during cold pressor test

Tests for Parasympathetic nervous system:

- Response of heart rate (HR) to standing
- Response of HR to deep breathing
- Heart rate response to Valsalva maneuver

Orthostatic Standing Test (OST)

The heart rate during initial 30 seconds after active standing from supine position is measured. Continuous heart rate recording demonstrate that the heart rate peaks at 15th beat, starts slowing at 20th second reaching a minimum at 30th beat.

When the subject assumes the erect posture from supine position, pooling of blood towards the lower limbs occurs due to gravity causing a decreased venous return which in turn reduces the cardiac output and arterial pressure that is sensed by the arterial baroreceptors which results in baroreceptor unloading and a consequent vagal withdrawal producing an instantaneous increase in heart rate, which peaks around 15th beat after which the blood pressure returns to normal level resulting from baroreceptor mediated slowing of heart rate, which is evident around the 30th beat (Borst et al 1982)[73].

This test provides an estimate of the cardiac parasympathetic control as the changes in heart rate are largely mediated by parasympathetic withdrawal and activation reflecting the changes in baroreceptor afferent traffic.

Deep Breathing Test (DBT)

Heart rate variability during respiration which is known as sinus arrhythmia results from the influence of afferent vagus on the medulla by reflex feedback loops, mediated by stretch receptors located in the lungs, chest wall, heart, blood vessels and by the respiratory centers.

It decreases with age and increases at slower respiratory rate at around 5-6 respiration/min. The changes in the baroreceptor responsiveness during different phases of ventilatory cycle provide additional modulation of heart rate.

The impulses from the stretch receptors in the lungs during inspiration cause vagal inhibition and the increased venous return due to fall in intrathoracic pressure causes the stimulation of arterial stretch receptors, which produce vagal inhibition and further spill over of impulses from respiratory center into the adjacent vagal motor neurons causes their inhibition leading to tachycardia. Conversely, expiration decreases the venous return and heart rate slows. After cooling of the vagus nerve or by atropinization it is documented that the reflex response is abolished (Fouad et al, 1984)[74]

Valsalva maneuver:

This procedure evaluates the function of the baroreceptors. This test is done by a forced voluntary expiration of a subject against resistance. It consists of four phases. Phase I- raise in transthoracic pressure, transient elevation in blood pressure and decrease in heart rate. Phase II- Reduced venous return resulting in low stroke volume, ultimately ends in reduced blood pressure and compensatory raise in heart rate. Phase III- End of expiration resulting in further reduced blood pressure due to pulmonary vascular expansion and heart rate increases. Phase IV– Baroreceptor activation, abrupt raise in blood pressure and bradycardia.

Fall of BP at the beginning of phase II should not exceed 21 mmHg and at the end of phase II or in phase III it should return to baseline values (Agnieszka Zygmunt et al, 2009)

"Valsalva Ratio (VR) is derived from the longest RR interval in phase IV divided by the shortest RR interval in phase II and at the very beginning of phase III". VR <1.21 is considered abnormal. Valsalva ratio reflects parasympathetic activity, whereas variations in blood pressure are a measure of sympathetic function.

Isometric Handgrip Test (IHG)

Sustained muscle contraction causes increased blood pressure and heart rate as a result of exercise reflex, which increases the sympathetic and reduce the parasympathetic activity. The heart rate changes by parasympathetic cholinergic

function and blood pressure changes are regulated by sympathetic adrenergic function. (Textbook of Clinical neurophysiology, Misra 2nd edition)[75]

Cold Pressor Test (CPT)

Immersion of hand or foot in ice cold water causes motor reflex activation, leading to elevation in blood pressure and cardiac output, stimulated by cutaneous pain receptors. Raise in vascular resistance leads to elevation of blood pressure due to enhanced sympathetic activity (Victor et al, 1987)[76]

Resting Heart Rate Variability test:

Studies in last 30 years have shown a great significance between autonomic nervous system and cardiovascular morbidity, including sudden death in arrhythmias (Levy et al, 1994)[77].

The duration of cardiac cycle of all heart beats occurring in one minute, even under resting condition is not the same. There is spontaneous beat to beat variability of RR interval in milliseconds which is known as Heart rate Variability (HRV). The reason for normal fluctuation of heart rate is respiratory arrhythmia, baroreceptor reflex, circadian rhythm and thermoregulation.

Hon and Lee in 1965[78] recognized that, alterations in HRV preceded before any notable changes occurred in heart rate as such in cases of foetal distress.

In 1985, Ewing et al[79] introduced simple test for short term study of RR difference to detect diabetic neuropathy

In 1981, Akselrod et al[80] introduced first power spectral analysis of heart rate fluctuation for quantitative evaluation.

The higher the HRV, the quicker and more flexibly the heart adapts to external and internal influences and the better the organism react to the environment. Higher heart rate variability reflects optimal cooperation between sympathetic and parasympathetic nervous system.

Depressed HRV means depressed activity of the autonomic regulatory function and the ability to maintain the homeostasis. It primarily means that heart rate is monotonously regular. A low HRV, indicates a reduced capacity for adaptation and may suggest serious health impairment (Sampler MB et al, Lele AS et al, 1980)

HRV changes might be a first sign of distress, reflecting involvement of more energy dependent sympathetic system. The decrease in biological signals variability is a warning sign of a homeokinetic self regulation loss. HRV can reflect changes in body stress, while other physiological parameters are still in "normal" accepted ranges.

Heart rate variability (HRV) is a measure of the respiratory sinus arrhythmia. Normally the heart rate accelerates during inspiration, and decelerates during expiration. This normal phenomenon decreases with age and also under stressful conditions. Human studies on blocking the parasympathetic system using atropine (Berntson et al, 1994) indicate that HRV is a function of parasympathetic activity.

Figure- 3. Sinus arrhythmia

The resting Heart rate variability is one of the non invasive tests to evaluate the integrity and functional state of ANS. Animal studies have shown that HRV is considered a marker of vagal activity (Brouha and Nowak, 1939).

It was proposed that the heart rate variability is a non invasive cardio graphic marker reflecting the activity of sympathetic and vagal component of ANS on sinus node of the heart (Stein P.K et al, 1994)

In 2004, Juan Sztajzel et al, stated that "heart rate variability has emerged as a simple non-invasive method to evaluate sympathovagal balance at the sinoatrial node among the different available noninvasive techniques for assessing autonomic status"[81].

HRV analysis being highly complex, "European society of cardiology and North American society of pacing and electrophysiology" have contributed a Task Force for developing appropriate standards. The guide lines and recommendations of ***Task Force, 1996*** [82]are followed in this study.

Measurement of HRV:

In this review the term HRV actually means the variability of RR intervals which is the interval between consecutive R peaks. In 1983, Persson et al[83] computed that the RR interval variation (RRIV), as a measure of the heart rate variability and one of the simplest and reliable test used for the evaluation of the autonomic functions of the heart.

In HRV analysis either the heart rate as a function of time or the intervals between successive QRS complexes need to be determined. Using the ECG signal it is possible to detect the regular pattern of beats using specially designed software algorithms. Such an algorithm is able to process the recording signal and determine with some precision when each beat is occurring.

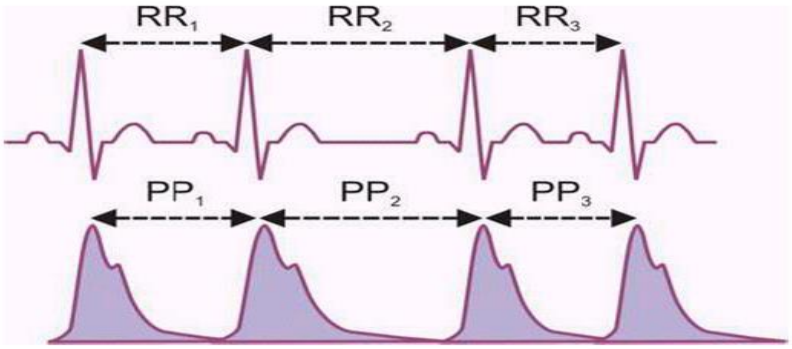


Figure -4. RR interval

Analysis of HRV:

Two broad categories of measures to standardize physiological and clinical studies were defined by the Task force, 1996.

- ***Time domain methods***
- ***Frequency domain methods***

HRV can be assessed either for a short term of 5 minutes or for a long term of 24 hours recording. Frequency domain methods should be preferred for short term recording and Time domain methods are preferred for long term recording (Task force, 1996).

Time domain methods:

Time domain measures are the simplest to calculate. By a continuous ECG record the heart rate at any given point in time and the interval between successive normal complexes is determined. Each QRS complex is detected and the so called NN interval that is all intervals between adjacent QRS complex resulting from sinus node depolarization or the instantaneous heart rate is determined. Time domain measures include the mean normal- to- normal (NN) intervals during the entire recording and statistical measures of the variance between NN intervals. In practice, RR and NN intervals usually appear to be same (Task force, 1996).

The majority of time domain metrics are statistical methods. It should be calculated over a specific and fixed period of time, or epoch, either short term for 5 minutes or long term for 24 hours to carry any significance.

The SDNN is the standard deviation of these Normal to Normal intervals which are simple variable and expressed in milliseconds. This is equal to square of variance which is equal to total power of variance. Significant results are achieved

only by comparing SDNN values which may be unreliable if they are calculated over too short epochs. SDNN reflects the variability in the period of recordings. As SDNN gets reduced HRV gets reduced.

Mean HR is the Mean of the selected RR interval and Mean RR is the Mean of the selected RR series.

Frequency Domain Methods:

Power spectral density (PSD) analyzer gives the fundamental information's of how the power (variance) distributes as a function of frequency. By proper mathematical algorithms, an estimate of true power spectral density can be obtained. The commonly employed methods are

- ***Non parametric methods***
- ***Parametric methods***

In non parametric method, Fast Fourier Transform (FFT) is used as algorithm. This is very simple and has a high processing speed. The Power Spectral Component has recordings ranging from 4-400 MHz. The result is shown on a power spectrum, which shows a breakdown of all the frequencies (oscillations) contained in each epoch. The spectrum is calculated over the same epoch duration as the SDNN metric (long or short term) and the relative power or total power for selected sub-bands of the whole spectrum is computed.

Frequency domain Power spectral density (PSD) analysis provides the basic information of how power distributes as a function of frequency. The PSD is

analyzed by calculating powers and peak frequencies for different frequency bands. The three main components that are distinguished in a spectral calculation for short term ECG recording are:

Components		***Frequency range***
Very low frequency	VLF	0-0.04Hz
Low frequency	LF	0.04-0.15 Hz
High frequency	HF	0.15-0.4 Hz

High frequency power (HF, 0.15-0.4Hz):

This power spectral oscillation is seen only for parasympathetic nervous system. HF power is mostly influenced by processes modulating gas exchange efficiency, respiratory sinus arrhythmia (RSA) and activity from the Vagus nerve. This is specially blocked by parasympatholytic drugs. Even 1 minute ECG recording is enough

Low frequency power (LF, 0.04-0.15Hz):

This is an indicator for more sympathetic than parasympathetic modulation. LF frequencies show activity of the baroreflex function which maintains blood pressure and of the sympathetic system. This needs minimum of 2 minutes ECG recording.

Very low frequency power (VLF, 0-0.04Hz):

The physiological explanation of the VLF component is much less defined and the existence of a specific physiological process attributable to these heart period changes might even be questioned. It is not much relevant in short term (5 minutes) ECG recordings.

Normalization of units:

LF (n.u) and HF (n.u) are the normalization of the powers which gives near 100% values of the sympathetic and parsympathetic events. They are calculated as follows:

LF (n.u) = LF power/(LF + HF power) or LF power / TP-VLF

HF (n.u) = HF power/ (LF+HF power) or HF power / TP-VLF

Total power = LF+HF power

LF/HF ratio:

This has been used as a index of global sympatho-vagal balance. Measurement of LF, HF is made in normalized units (Mallinai A et al, 1991)[84]. The distribution of LF and HF are not fixed but vary in relation to changes in the autonomic modulation of heart period.

Clinical Uses of HRV:

HRV is the non-invasive simple test for measuring both cardiovascular and non cardiovascular autonomic function. HRV analysis is a predictor of risk for myocardial infarction, arrhythmias and diabetic autonomic neuropathy.

HRV analysis is used to find out the functional disorders earlier. It is used to evaluate the effectiveness of treatment and prognosis and to confirm the effect of stress relaxation program (meditation, massage, light therapy, exercise). HRV is used for exercise training in sports physiology

Leptin:

Leptin, is one of the most important adipocytokine produced by the adipocytes and is considered to be an appetite suppressor peptide (satiety factor) named "leptin" after the Greek word "leptos" for thin. It is a 16 kDa protein product of Ob gene consisting of 167 amino acids. It was originally discovered by JM Friedman through positional cloning of *ob/ob* mice, a mouse model of obesity found at Jackson Laboratories. Since then it is one of the most important central and peripheral signals for the maintenance of energy homeostasis (Friedman JM et al, 1998)[85].

The "Ob (Lep)" gene, is located on chromosome 7 in humans, Ob stands for obese, Lep for leptin. Specific leptin receptors (Ob-R) are expressed in the brain as well as in peripheral tissues. Ob/ob mice, which lack leptin and db/db mice which

lack leptin receptors are obese. Human beings with a homozygous mutation in the leptin gene or the leptin receptor have morbid obesity.

Mechanism of action of Leptin:

Leptin Receptors (Ob-Rs):

There are six isoforms of leptin receptors namely Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, Ob-Re and Ob-Rf, and are closely related to Class I cytokine receptor family. Ob-Ra and Ob-Rb represent the dominant isoforms in the heart while the other receptors are expressed at low levels (Fei H et al, 1997)[86]. The Ob-Ra isoform which is short leptin receptor isoform, plays an important role in transporting leptin across the blood–brain barrier. ObRb isoform which are the long leptin receptor isoform are present in 'the hypothalamus, an important site for the regulation of energy homeostasis and neuroendocrine function' and mediate signal transduction. Ob-Re is the secreted form that binds circulating leptin, regulating the concentration of free leptin[87].

Leptin signaling:

It is observed that Ob-Rs activate mitogen-activated protein kinase (MAPK) pathways, Janus-activated kinase (JAK), insulin receptor substrate, signal transducers and activators of transcription (STAT), of which the most predominant is the JAK/STAT pathway (Vaisse and Halaas et al, 1996)[88]. Leptin receptors on ligand binding undergo homooligomerization (Nakashima K et al,

1997)[89]. Then it binds with Janus Kinase JAK2 primarily (Kloek Cet al, 2002)[90]. This leads to JAK2 autophosphorylation and phosphorylation of tyrosine kinases involved in intracellular cytokine signaling. Binding of members of Suppressors of cytokines signaling family (SOCS3) to tyrosine or receptor janus kinase complex mediates negative feedback on leptin signaling (Dunn SL et al, 2005)[91]. Only Ob-Rb contains STAT-binding site (Bjorbeck C et al)[92].

The long-form leptin receptor (Ob-Rb) signaling through JAK2/STAT3 is required for control of feeding and energy expenditure to maintain normal energy homeostasis. Mutations of this gene result in the obese phenotype of the *db/db* mouse and the Zucker rat (Zhang Yet al, 1994)[93]. The soluble leptin receptor isoform Ob-Re may oppose leptin transport by decreasing surface binding and endocytosis of leptin(Bates SH et al, 2003)[94]

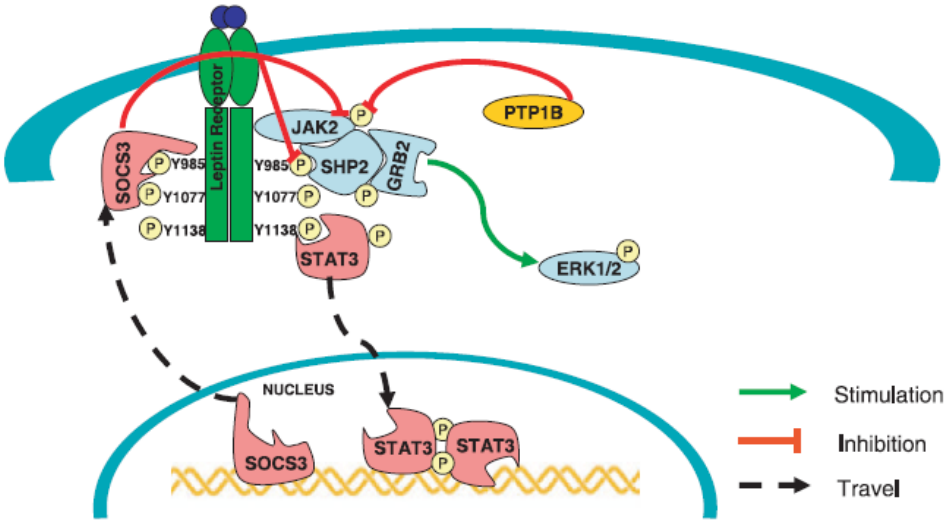


Figure- 5 Leptin signaling pathway

Disruption of leptin signaling in the hypothalamus leads to obesity and confirms the central role of leptin in the maintenance of energy balance (Cohen P et al, 2001)[95]. In addition to its action on CNS, through its peripheral receptors affect many systemic processes, such as immunity (Lord GM et al, 1998)[96], reproduction (Margetic S et al, 2002)[97], and cardiovascular functions (Rahmouni J and Haynes WJ et al)[98].

Leptin secretion is pulsatile with a prominent diurnal variation with higher levels in early morning and evening. Circulating leptin levels correlates with the amount of energy stored in fat and changes in caloric intake (Licinio J et al, 1998)[99]. Women have greater leptin concentrations than men. Leptin concentration in women is higher in the luteal phase of the menstrual cycle (Ludwig M et al, 2000)

Leptin Resistance or Tolerance:

Hyperleptinemia is seen in many genetic variants including the Lys109Arg or Gln223Arg mutation in the leptin receptor (LEPR) gene. Mutations of some other genes including Pro-opio melanocortin (POMC) and the melanocortin 4 receptor (MC4R) also result in an obese phenotype with associated neuroendocrine dysfunction.

In obesity Leptin transport across the blood-brain barrier is impaired. This is partially due to saturation of the transporter as a result of hyperleptinemia and a subsequent decrease in transport activity (Van Rossum C et al 2003).

Endoplasmic reticulum stress is one of the proposed causation for development of leptin resistance. Humans and animals with obesity and diabetes have elevated endoplasmic reticulum stress in adipose tissue, liver and pancreatic cells which decreases signaling of leptin receptors in hypothalamus. (Sharma NK et al 2008).

Role of Leptin in Food Intake and Energy Homeostasis:

Leptin plays a significant role in maintenance of energy homeostasis, metabolism, and neuroendocrine function. Level of circulating leptin is proportional to amount of adipose tissue store. It is an indicator for energy reserves and directs the central nervous system to adjust food intake and energy expenditure accordingly.

Leptin acts immediately on the brain especially hypothalamus to regulate appétite via ObRb-receptor binding activateing a complex neural circuit via melanocortin pathway comprising of anorexigenic and orexigenic neuropeptides to control food intake (Kelesidis et al). Arcuate nucleus in the hypothalamus sends output to Lateral nucleus which is the feeding centre, and Ventromedial centre which is the satiety centre.

Arcuate nucleus contains two types of neurons of which the first group co-expresses Neuropeptide Y (NPY) and Agouti related Peptide (AgRP) which are orexigenic neuropeptides that is stimulatory to feeding centre and inhibitory to satiety centre. The other group co-expresses POMC (Pro-opiomelanocortin) and CART (Codeine and amphetamine regulated transcript) which are anorexigenic neuropeptides that is stimulatory to satiety centre and inhibitory to feeding centre. Leptin stimulates POMC/CART while it inhibits NPY/AgRP group thus suppressing appetite[100].

Leptin increases sympathetic outflow and activates brown adipose tissue thermogenesis. Leptin levels fall rapidly in response to fasting. It also slows down metabolic rate by decreasing thyroid hormone levels, mobilizes energy stores, and decreases insulin-like growth factor-1 (IGF-1) level (Ahima RS et al, 1996).

Regulation of Insulin Sensitivity:

Leptin improves insulin sensitivity by reducing plasma glucagon and growth hormone levels. Leptin similar to insulin increases fatty acid oxidation and skeletal muscle glucose. Leptin acts through insulin-signaling pathways, particularly phosphatidylinositol 3-kinase (PI3K) and 5-AMP-activated protein kinase (AMPK) activation.

Leptin acts as a signaling molecule released from the adipocytes acting on the endocrine pancreas controlling the insulin secretion in accordance with the

energy stores of the body, forming "Adipo-insular axis" which is a classic endocrine feedback loop.

Leptin decelerates lipid deposition in adipose tissue promoting energy expenditure (Minokoshi Yet al 2002)[101]. Leptin has an insulin promoter activity and also effectively inhibits glucagon secretion from pancreatic cells.

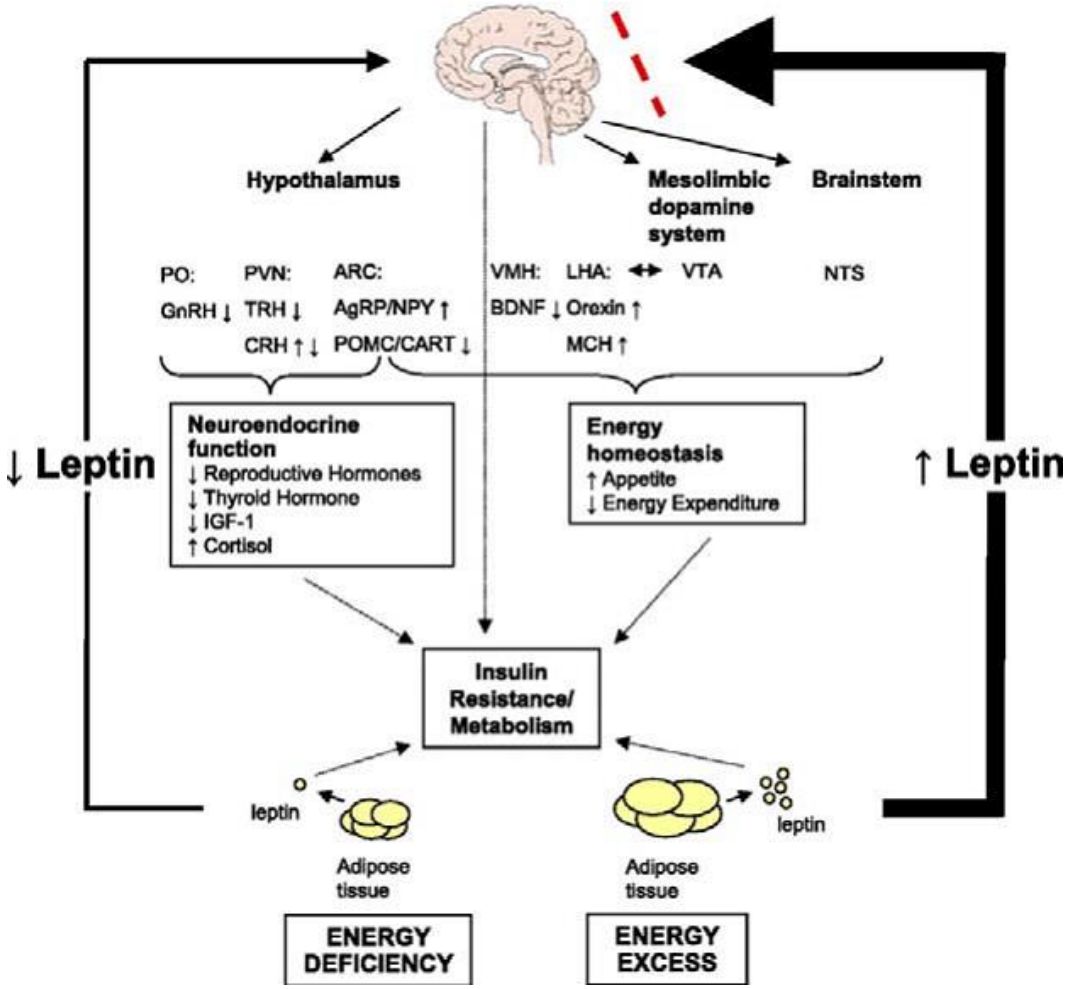


Figure - 6 Role of Leptin

Regulation of Neuroendocrine Function:

Leptin is found to be an important mediator of the hypothalamo-pituitary-gonadal axis. LH pulsatility and testosterone secretion was blunted in healthy lean men on fasting. Leptin replacement brought back these hormones to the prefasting levels. Similar effects were observed in normal weight women. Infertile ob/ob mice became fertile on exogenous leptin administration.

Leptin regulates reproductive function by activating neurons that project afferent input to GnRH neurons in the preoptic area and other hypothalamus suggesting that leptin may mediate the reproductive axis through regulation of kiss peptins, products of the Kiss1 gene, as well as dynorphin and neurokinin B. (Roux N et al, 2003). Leptin threshold of 3ng/mL is necessary to signal the brain the message that energy stores in adipose tissue are adequate to bring pregnancy to term.

Role of Leptin in Obesity:

Elevated levels of leptin and hyperphagia are features of adult onset obesity. Leptin a potent inhibitor of food intake is expected to decrease insulin levels. This paradox is explained by the 'selective leptin resistance'. Hyperleptinemia contributes to increased sympathetic activity and arterial pressure in obesity, with a resistance to the metabolic actions of leptin like satiety and weight reduction. This may be because of a suppressed central action due to the

saturation of receptors with a persistent peripheral action or due to leptin resistance.

Patients with congenital complete leptin deficiency due to homozygous leptin gene mutations develop extreme obesity from birth and have distinct neuroendocrine abnormalities, including hypogonadotropic hypogonadism with failure to reach puberty.

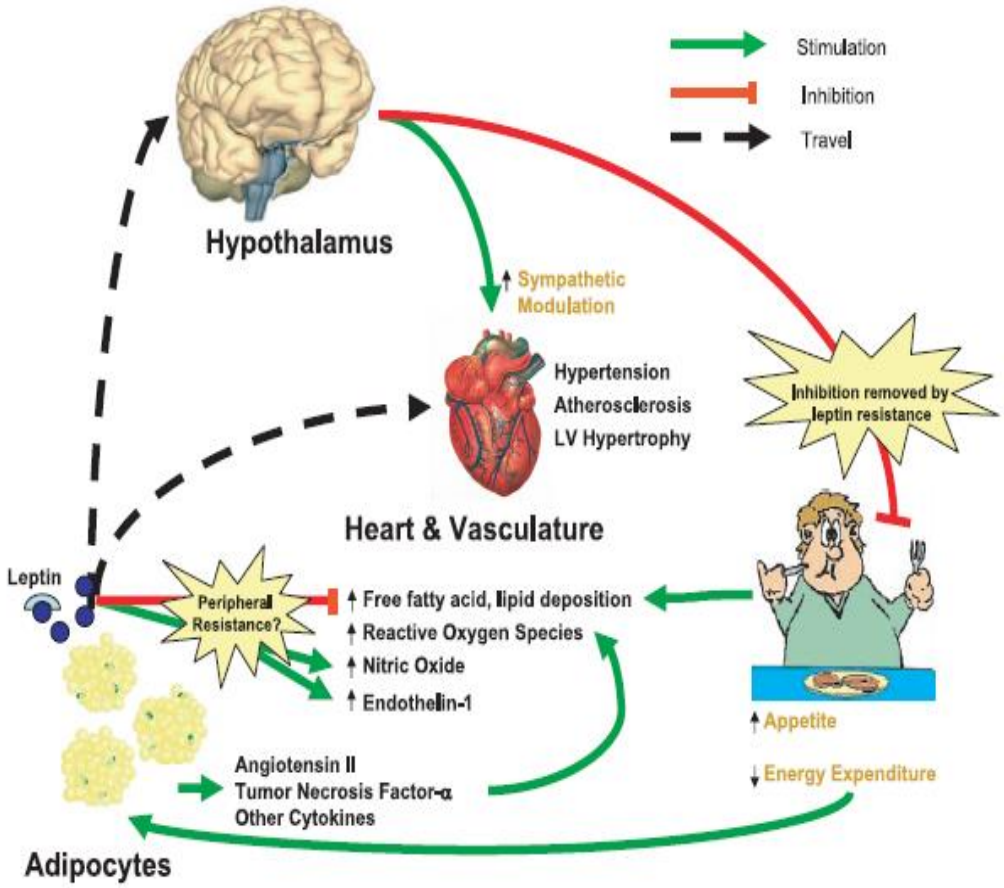


Figure- 7 Role of Leptin in Obesity

Effect on Cardiovascular system:

Plasma leptin concentration is a novel, independent risk factor for coronary events (Michael Wallace et al, 2001). Hyperleptinemia is associated with lower arterial distensibility, promotes angiogenesis and enhances the calcification of vascular cells and potentiates the pro-thrombotic platelet aggregation through a leptin receptor-dependent mechanism thus increasing the coronary risk. (Sierra-Honigmann et al, 1998)[102]

Leptin stimulates the sympathetic system, increases energy expenditure and leptin infusion is found to increase the catecholamines levels and thus produce an increased heart rate and blood pressure. A negative feedback loop is formed between leptin and the sympathetic nervous system by the catecholamines like norepinephrine, epinephrine and isoprenaline that have an inhibitory effect on leptin synthesis (Eikelis et al, 2003).

Leptin exerts direct, non neural effects on cardiovascular function. Leptin receptors or their mRNA have also been detected in the heart and endothelium of blood vessels (Ren J et al). Leptin produces a nitric oxide dependent arterial vascular relaxation with the chloride ion being an important regulator in leptin-induced endothelial NO release (Kimura et al. 2000) which serves as a counter-balance for the enhanced sympathetic activity in response to leptin.

The higher heart rate in the hyperleptinemia will impose a greater myocardial workload and therefore predispose the heart to pathological changes.

Leptin causes an increased oxygen consumption and decreased cardiac efficiency (Atkinson et al, 2002)[103].

Some of the actions of leptin on the cardiovascular system and the pathways involved are enlisted below.

Leptin Action	**Pathways Involved**
Liporegulation	Increased fatty acid oxidation
Development of hypertension	Increased catecholamine release, increase in preserved renal sympathetic nerve activity, Nitric oxide
Development of atherosclerosis	Endothelial cell Nitric oxide and reactive oxygen species (ROS) production proliferation and hypertrophy, platelet aggregation, proinflammatory cytokine production
Impaired cardiomyocyte contractility	JAK/STAT, NO-cGMP, G protein–coupled receptor, adenylate cyclase, Endothelin -1 (ET-1), NAPDH, ROS
Increase in size of cardiomyocyte	Endothelin-1, Reactive Oxygen Species
Enhanced cardiomyocyte mitosis and proliferation	ERK1/2, p38, PI3K, peroxisome proliferator-activated receptor-α

Protection from ischemia/reperfusion injury	PI3K, ERK1/2, Nitric oxide, Reactive Oxygen species
Deficiency leads to increased rate of cardiomyocyte apoptosis	p38, caspase-3, DNA damage, Angiotensin II

Chethan et al, in 2012[104], conducted a comparative study of heart rate variability in normal and obese young adult males which include 50 controls and 50 cases based on BMI. There was a significant reduction in SDNN, E/I ratio, HF nu and increase in LF nu and LF/HF in cases when compared to controls suggesting that there was a significant reduction in parasympathetic activity and increase in sympathetic activity. There was a shift in sympathovagal balance towards sympathetic predominance among obese males in contrast to normal males.

Mehmet Erkan Altuncu et al in 2012[105], conducted a study of short term analysis of heart rate variability in 66 obese children and 40 healthy controls and found that high frequency parameter values were lower in obese compared to controls with p value of 0.046 and low frequency by high frequency ratio was found significantly higher $p < 0.001$. He also found insulin resistance in 50% of obese, dyslipidemia in 59%, hypertension in 27% and metabolic syndrome in 39% of patients in obese group.

Rajalakshmi et al in 2012[106], conducted a study of heart rate variability on Indian obese young adults. The study included 60 young adults of whom 30 were obese and 30 normal weight groups. It was found Mean HR increased which they suggested to be due to relative sympathetic dominance and demonstrated a decrease in parasympathetic nerve activity. HRV indices significantly associated with obesity indices. It was also suggested that time and frequency domain analysis of HRV in obese shows imbalance in autonomic neural activity of heart.

Masood Y Al Maskari et al and Adel A Alnaqdy et al in 2006[107], conducted a correlation study between serum Leptin levels, Body mass index and obesity in Omanis which included 35 obese and 20 non obese healthy subjects. There was a significant difference in serum leptin between the obese and the control group with significantly positive correlation between leptin levels in obese subjects with weight, body fat percentage and BMI.

AIM AND

OBJECTIVES

3. AIM AND OBJECTIVES

Aim:

To evaluate Cardiovascular Autonomic Functions in Young Obese individuals and to estimate their serum leptin levels

Objectives:

- To assess and compare the cardiovascular autonomic functions in young obese individuals and the normal individuals
- To estimate the serum leptin levels in young obese individuals and compare it with that of normal individuals
- To correlate the serum leptin levels with HRV indices, body mass index and waist circumference in young obese individuals
- To correlate the HRV indices with body mass index and waist circumference in young obese individuals

MATERIALS AND METHODS

4. MATERIALS AND METHODS

This study was conducted at Madras Medial College, Chennai, in the Institute of Physiology and Experimental Medicine, after obtaining the Institutional Ethical Committee clearance. The study was conducted on a group of 50 young obese individuals with BMI ≥ 25 in the age group of 18 to 25yrs of both sexes and 50 age matched normal individuals with BMI 18.5 to 24.9 as controls from October, 2014 to August, 2015. The study included equal number of males and females. 25 males and 25 females in the control group and 25 males, 25 females in the young obese group that is a total of 100 participants were included in the study.

4.1. Selection of cases:

Fifty young obese individuals of both sexes in the age group of 18-25yrs with body mass index ≥ 25 were selected from patients attending the outpatient department of endocrinology at 'Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai – 3'

Height and weight of the subjects were measured and BMI calculated using Quetelet's index, named after Belgian astronomer Quetelet

$$\textbf{BMI} = \frac{\textbf{Weight in Kg}}{\textbf{Height in meter}^2}$$

4.1.1. Inclusion criteria:

Age group of 18 – 25 years

Both sexes

Body mass index ≥ 25

4.1.2. Exclusion criteria:

Pregnancy, Post- partum period

Systemic disorders like diabetes and hypertension

Respiratory, hepatic, neurological and cardiovascular disorder

Any medical illness (i.e. respiratory and heart failure and renal disease)

Hypothyroidism, Anaemia

Neoplasia, any secondary infections

Use of antidiabetic, antihypertensive, antiobesity drugs

Use of glucocorticoids, oral contraceptives or any hormonal therapy within the previous 6 months

Any infectious disease

Fifty subjects fulfilling the above criteria were selected and they were assigned as the study group

4.2. Selection of controls:

Fifty normal individuals with body mass index 18.5 - 24.99 of both sexes were chosen from the general population and they were assigned as the control group.

4.3. Study design:

Case - Control Study

4.4. Materials required:

Niviqure Ambulatory Digital ECG Recorder (INCO)

Sphygmomanometer

Hand grip Dynamometer

Cold water basin that contain water at temperature of 4-6°C

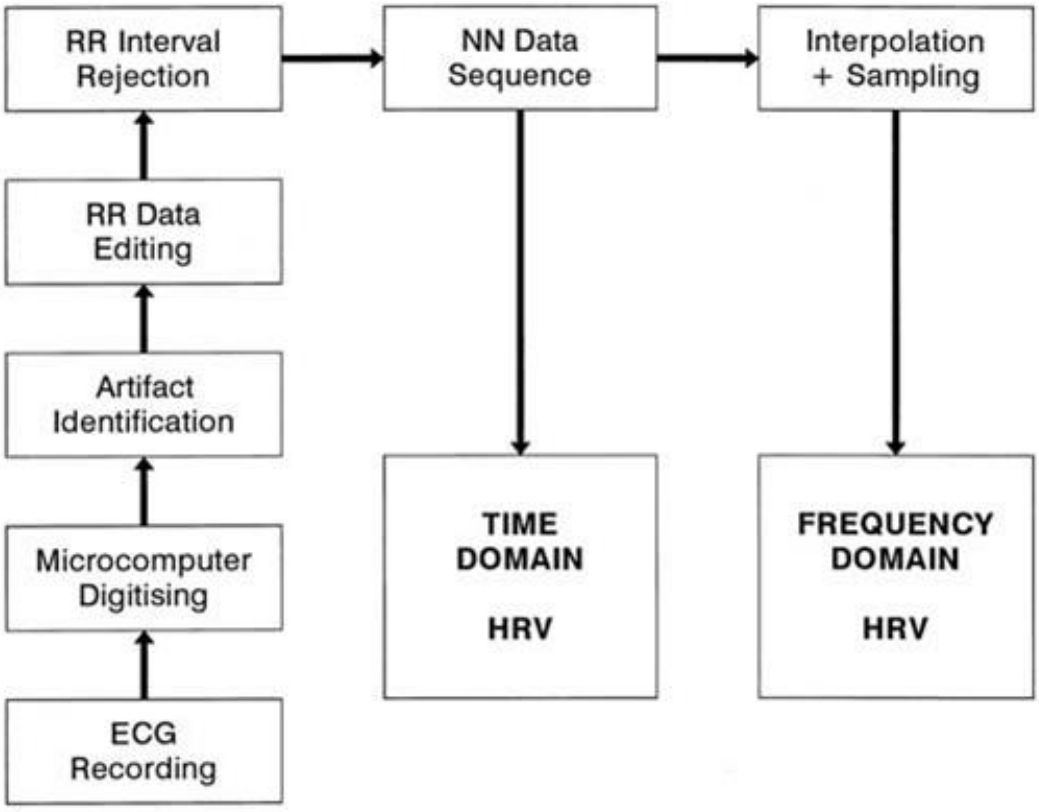
4.4.1. Description of the instruments:

Niviqure Ambulatory Digital ECG Recorder:

Niviqure ECG recorder is a digital, solid state, stand alone, multi load computerized recording system. It is designed to acquire ECG, analyze it and store

the ECG data over long hours. The data is acquired and it is stored in flash memory. The data can be later downloaded and further analysis done. The transfer of data from the flash memory module to the computer is via an interface RS233C which is a compatible module.

Niviqure has powerful processing software for online ECG study, for storage of data and off line data replay and study. The data can be transferred to other software for statistical analysis and Fast Fourier Transformation (FFT) analysis. The picture given below summarizes the individual steps followed while recording and processing the ECG data for analysis of HRV.



4.5. Methodology:

After selection of subjects, an informed, written consent was obtained from the study group and also the control group.

After taking a detailed history, their weight in kilograms and their height in meters were measured and their BMI was calculated as weight/height in meter square. Their WC and HC were measured and their WHR was calculated.

$$\textbf{WHR} = \frac{\textbf{Waist circumference}}{\textbf{Hip circumference}}$$

A thorough general as well as systemic examination was done. 5ml of blood samples were collected simultaneously for determining their leptin level.

The procedures were explained to the subjects and the fifty study group and fifty controls were subjected to a battery of autonomic function tests including resting heart rate variability as Ewing et al has described. These tests examine the HRV and blood pressure at rest and their response to some of the manouvers which are normal physiologic stimuli.

Many factors both internal and external can confound the results of autonomic function tests. These confounding factors should be controlled. The precautions that are taken while performing the autonomic function tests are as follows:

- The subjects should be relaxed and comfortable without any significant anxiety, and free from recent acute illness.
- The subjects were asked to empty the bladder before the test.
- Compressive garments which are uncomfortable are to be avoided.
- The autonomic function tests were carried out two hours after breakfast between 10 -12 AM.
- Caffeine, nicotine and alcohol should be avoided 2 hours before testing.
- The tests were performed in a quiet room , lighting subdued and with controlled, comfortable temperature ranging from 25-28° C
- Electronic gadgets were removed, mobile phones were switched off.
- The subjects were instructed about various maneuvers and allowed to practice these maneuvers.
- The subjects were made to rest quietly for a minimum period of ten minutes, without moving, in the awake and supine position.

Electrodes were placed in the following position after wiping the site with sprit.

Electrode	Position
Exploring electrode	Left shoulder
Exploring electrode	Right shoulder
Exploring electrode	Left subcostal
Reference electrode	Right subcostal

4.5.1. Resting Heart rate variability:

The total period of rest was increased to 30 minutes. During this resting period, ECG was acquired for five minutes (320 seconds), by a continuous recording which is required for short term ECG analysis. The ECG data is screened for any artifact. After editing it, the results were fed to HRV analysis software.

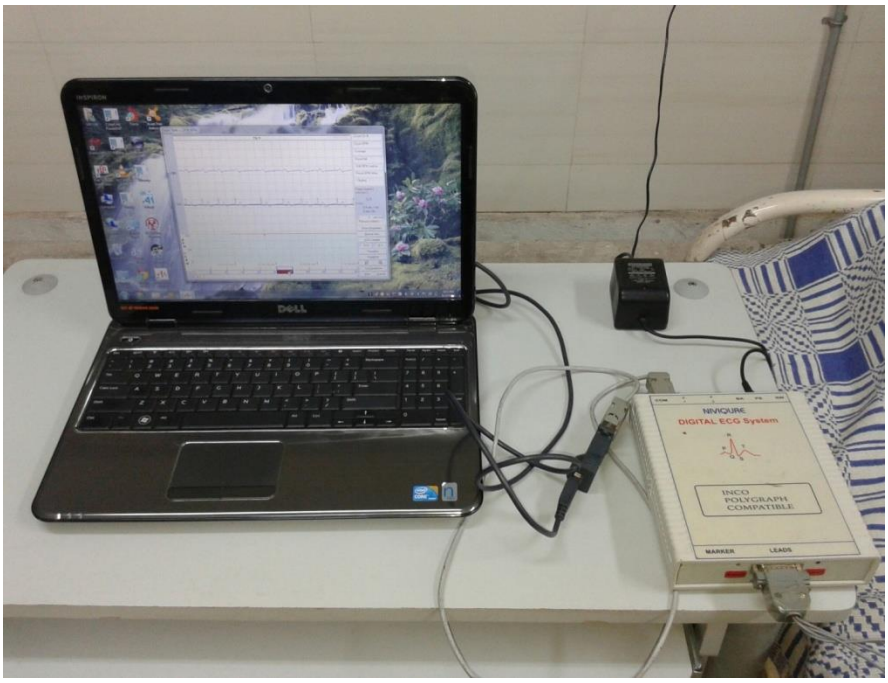
The analogue to the digital conversion of the resting ECG signal was done with sampling frequency of 1024/sec using AD converter. The converted ECG signal was analyzed under power spectrum using Fast Fourier Transformation (FFT) analysis.

SDNN, Mean HR, normalized Low frequency (LF nu), normalized High frequency (HF nu), LF/HF ratio were all estimated.

4.5.2. Orthostatic Standing Test:

The subject was allowed a minimum of ten minutes rest in supine position. Then the subject was allowed to stand immediately within a few seconds without any support by putting equal weight on both the legs, for a period of five minutes. Pulse rate and blood pressure was recorded after 1 minute & 3 minutes.

Photograph.1. Niviqure Ambulatory Digital ECG recorder



Photograph.2. Recording of Resting HRV

Measurement of R-R interval was done at the15th beat which was minimum and at 30th beat which was maximum.

$$\textbf{30/15 ratio} = \frac{\textbf{RR interval at 30th beat}}{\textbf{RR interval at 15th beat}}$$

The variations in SBP and DBP after 1 minute of standing were recorded.

The values are compared with normal values obtained from tcontrol group.

4.5.3. Deep Breathing Test:

The subject after being seated comfortably was asked to breathe deeply and slowly as per the verbal commands such that the subject inspires deeply for 5 seconds and expires slowly for a period of 5 seconds. Thus one cycle of inspiration and expiration lasts for 10 seconds. Six such cycles were performed lasting for about 1 minute.

The average of RR intervals which are long and short during expiration and inspiration respectively for 6 cycles was obtained and from this the Expiration/Inspiration ratio was calculated.

$$\textbf{Expiration/Inspiration ratio} = \frac{\textbf{Longest RR interval during expiration}}{\textbf{Shortest RR interval during inspiration}}$$

The values were compared with normal values of control group.

4.5.4. Valsalva Maneuver:

The test was performed after another 5 minutes interval of rest in sitting posture. A forced expiration was done to maintain 40 mmHg pressure in the mercury manometer for a period of 15 seconds during which ECG was recorded and ECG recording was proceeded after maneuver for a duration of 30 seconds. Valsalva ratio calculated.

$$\text{VR} = \frac{\text{Longest RR interval after strain}}{\text{Shortest RR interval during strain}}$$

4.5.5. Isometric Handgrip Test:

The patient was placed in supine and relaxed position and the blood pressure was recorded. A simple exercise using a hand grip dynamometer was performed by the subject, as instructed. The subject was asked to press the hand grip dynamometer with maximum force for few seconds and the same was repeated thrice. The highest reading was considered as Maximum Voluntary Contraction (MVC). Hand grid to attain 30% of MVC for duration of 5 minutes was done and blood pressure was measured in the opposite limb during the

Photograph.3. Orthostatic standing test

Photograph.4. Isometric Handgrip test

procedure and after the procedure**. Increase in DBP = DBP after procedure – Diastolic blood pressure at rest.**

4.5.6. Cold pressor test:

Immersion of hand or feet inside the cold water (4°) for 60- 90 seconds cause activation of sympathetic system via afferent pain and temperature fibers of skin. There is a raise in diastolic blood pressure which predicts sympathetic activity.

Results from all tests were statistically evaluated. Accordingly differences in autonomic functions in study and control group were assessed.

4.6. Assessment of Serum leptin levels:

4.6.1. Sample collection and storage:

Under strict aseptic precautions about 5ml of blood was collected by venepuncture from the subjects and was centrifuged. Serum was separated, serum samples were stored at -20° Celsius. The samples were then transported under cold chain maintenance to Central Research Laboratory at Tamil Nadu Dr.MGR Medical University, Guindy, Chennai, for performing the test. DRG Diagnostics

Photograph.5. Cold Pressor test

Photograph.6. Sample collection

Leptin (Sandwich) Elisa kit was used for assessing serum leptin levels. Appropriate national biohazard safety guidelines were followed.

4.6.2. Principle of the test:

The DRG Leptin ELISA kit is a solid phase enzyme linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a Leptin molecule. Subjects sample containing endogenous Leptin is incubated in the coated microtitre wells which are then coated with a specific biotinylated monoclonal anti Leptin antibody. A sandwich complex is formed. After incubation the unbound material is washed off and a Sreptavidin Peroxidase Complex is added for detection of the bound Leptin. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Leptin in the given sample

4.6.3. Reagents Required:

1. Microtiter wells Plate with Break Apart Wells coated with monoclonal anti-Leptin antibody

2. Standard (0-5) with concentrations 0-2-5-25-50-100 ng/ml

3. Control (Low and High)

4. Assay Buffer Leptin Calibrators

5. Antiserum – Monoclonal biotinylated anti-Leptin antibody

6. Enzyme Complex – Streptavidin conjugated to horseradish peroxidase

7. Substrate Solution – Tetramethylbenzidine (TMB)

8. Stop solution – 0.5M Sulphuric acid

9. Wash Solution

4.6.4. Other materials required

A microtiter plate calibrated reader (450 ± 10 nm)

Calibrated variable precision micropipettes

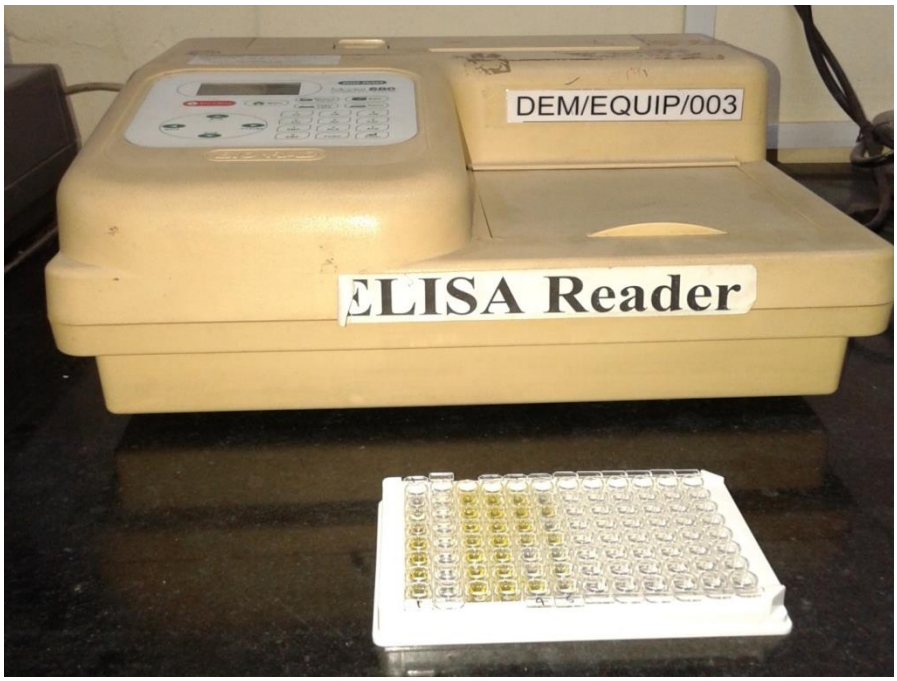
Distilled water, absorbent paper, timer, semi logarithmic graph paper

4.6.5. Assay procedure:

All reagents and specimens were allowed to come to room temperature before use. Standard controls or samples about 15 μL each were added with new tips that are disposable into corresponding wells that were labeled.

Photograph.7. DRG Leptin ELISA kit

Photograph.8. ELISA Reader, TN Dr.M.G.R.Medical University



Assay buffer about 100 µL was dispensed into each well. Then it was mixed for 10 seconds. It was then incubated for 2 hours at room temperature. Well contents were shaken out briskly. About 300 µL of wash solution was dispensed into each well to wash the wells. Wells were washed 3 times with diluted wash solution. Wells were stroke on absorbent paper to remove residual droplets.

Antiserum about 100 µL each was dispensed. Then it was incubated at room temperature for 30 minutes. Well contents were shaken out. Wash procedure repeated and wells were stroke on absorbent paper.

100 µL of Enzyme Complex each was added and incubated at room temperature for 30 minutes. Then contents were shaken out. Wash procedure repeated and wells were stroke on absorbent paper.

Substrate solution about 100 µL was dispensed and incubated at room temperature for 15 minutes. Stop Solution 50 µL each was dispensed to stop enzymatic reaction. Within 10 minutes using ELISA microtiter plate reader, absorbance was found at 450 ± 10 nm for all the wells.

4.6.6. Calculation of Results:

Absorbance (Optical density) values of standard controls and samples were calculated. With concentration on X axis and absorbance value on Y axis, plotting the mean absorbance got from each standard against its concentration, a standard

curve was constructed. Using this standard curve based on mean absordance value, corresponding concentration of each sample was found.

Normal values in lean men are 2 to 5.6 ng/ml and in lean women are 3.6 to 11.1ng/ml.

Using the Statistical Package for Social Sciences (SPSS) software version 21, all the data obtained from all the autonomic functions tests and serum leptin levels were analyzed.

RESULTS

5. RESULTS

Data obtained from conducting the Autonomic Function Tests and Serum Leptin levels were statistically analyzed. Mean and standard deviation of the variables were determined for the control and young obese groups. Independent (Unpaired) t test was employed for statistical analysis as the test of significance at 95% confidence interval.

*** P value < 0.05 was considered as significant**

**** P value < 0.01 was considered as highly significant**

*****P value < 0.001 was considered as very highly significant**

Comparison of physical characters among study groups:

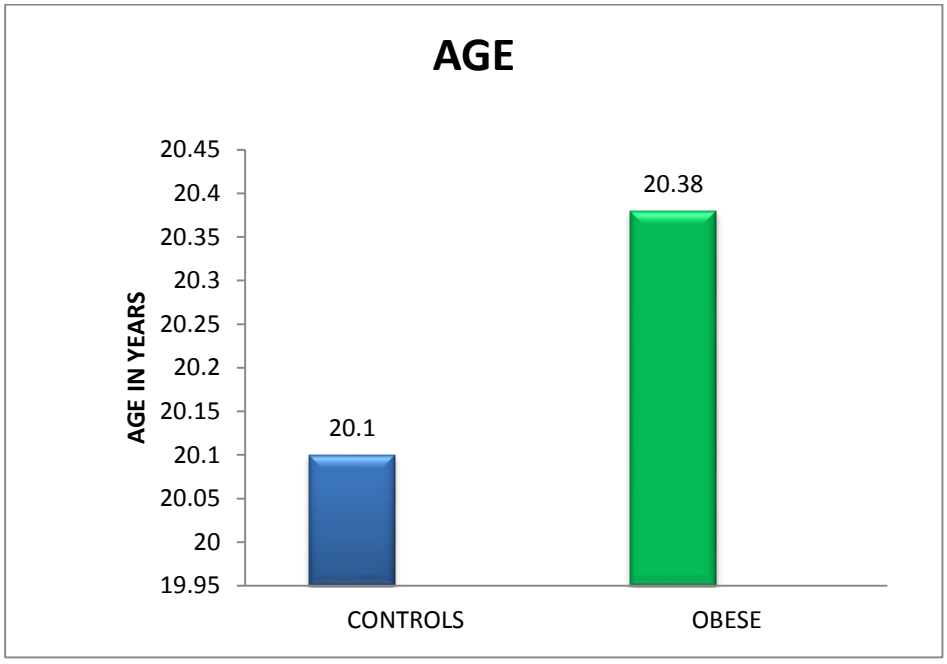
TABLE NO:1

COMPARISON OF AGE AND HEIGHT AMONG STUDY GROUPS

VARIABLE	STUDY GROUP	N	MEAN	SD	P-VALUE
AGE(years)	CONTROLS	50	20.10	1.05	0.4163
	OBESE	50	20.38	2.18	
HEIGHT(m)	CONTROLS	50	1.60	0.06	0.179
	OBESE	50	1.58	0.07	

Table No: 1 compares the age and height of the controls and obese groups. The P value of age and height were not significant.

GRAPH NO: 1 COMPARISON OF AGE AMONG STUDY GROUPS

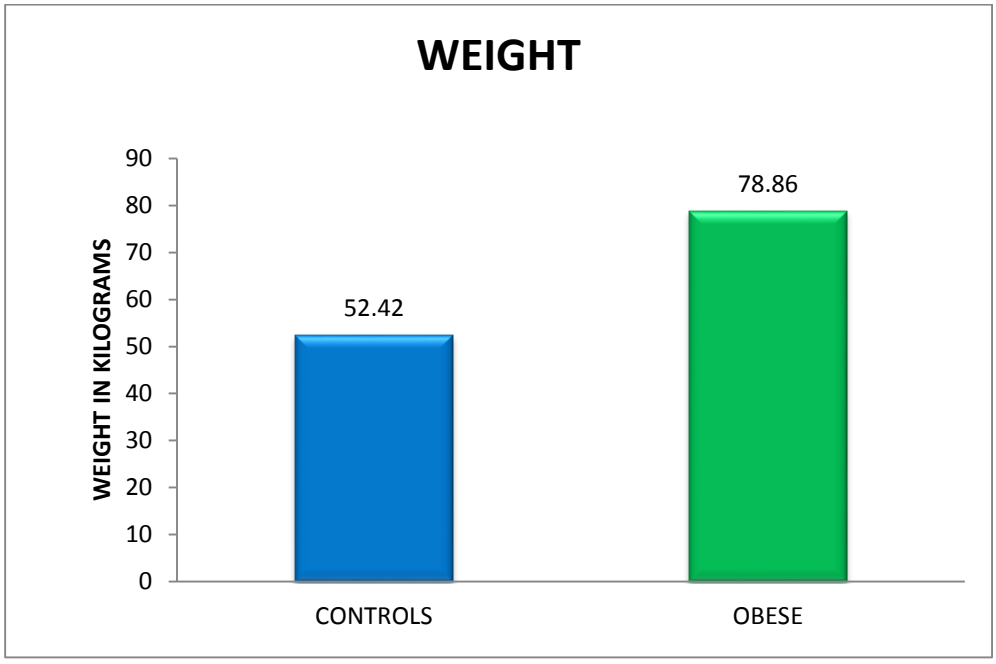


GRAPH NO: 2 COMPARISON OF HEIGHT AMONG STUDY GROUPS

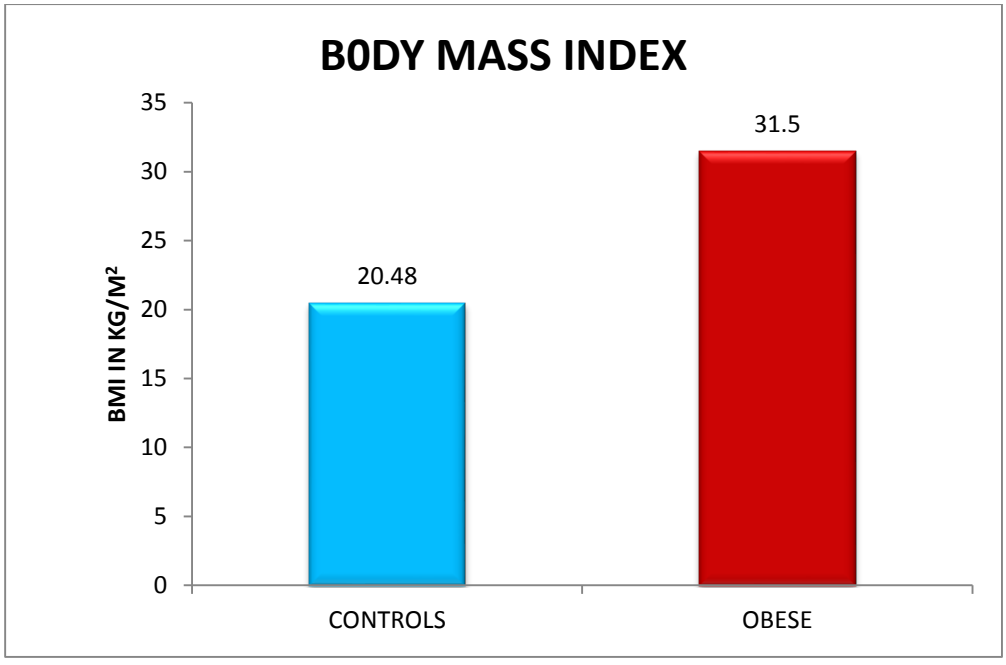
Comparison of obesity indices among study groups:

TABLE NO: 2				
VARIABLE	STUDY GROUP	MEAN	SD	P-VALUE
COMPARISON OF WEIGHT AMONG STUDY GROUPS				
WEIGHT(kg)	CONTROLS	52.42	2.58	0.000***
	OBESE	78.86	5.62	
***** very highly significant**				
COMPARISON OF BODY MASS INDEX AMONG STUDY GROUPS				
BMI	CONTROLS	20.48	1.09	0.000***
	OBESE	31.50	2.25	
***** very highly significant**				
COMPARISON OF WAIST CIRCUMFERENCE (WC) AND HIP CIRCUMFERENCE (HC) AMONG STUDY GROUPS				
WC (cm)	CONTROLS	75.26	1.91	0.000***
	OBESE	106.30	5.37	
HC (cm)	CONTROLS	93.90	4.0	0.000***
	OBESE	107.34	5.81	
***** very highly significant**				
COMPARISON OF WAIST HIP RATIO AMONG STUDY GROUPS				
WHR	CONTROLS	0.80	0.04	0.000***
	OBESE	0.99	0.02	
***** very highly significant**				

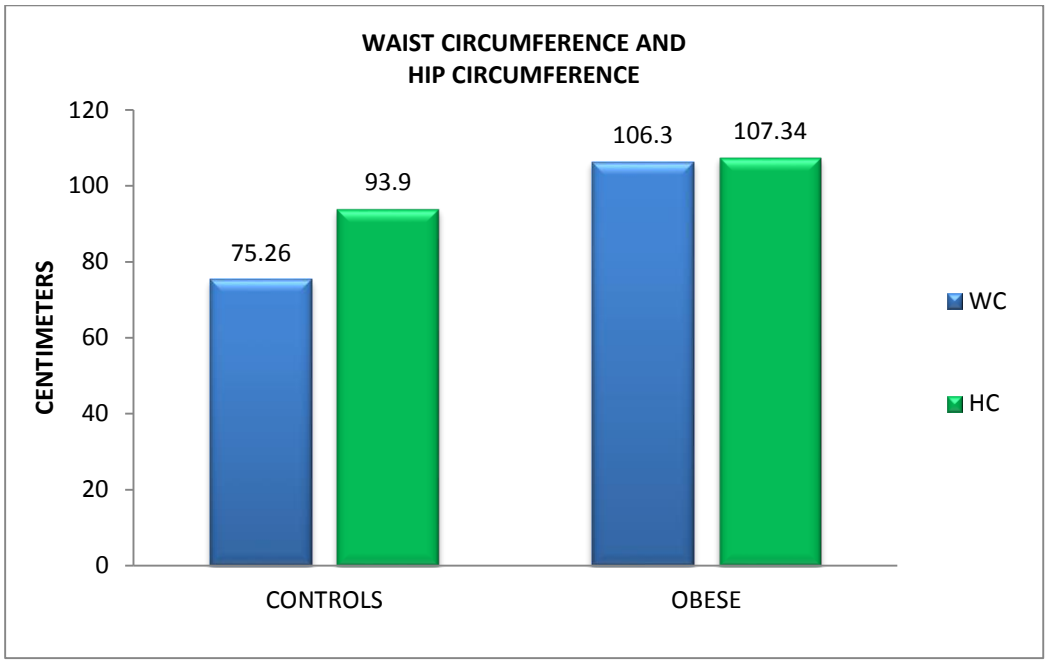
GRAPH NO: 3 COMPARISON OF WEIGHT AMONG STUDY GROUPS



GRAPH NO: 4 COMPARISON OF BODY MASS INDEX AMONG STUDY GROUPS



GRAPH NO: 5 COMPARISON OF WAIST CIRCUMFERENCE AND HIP CIRCUMFERENCE AMONG STUDY GROUPS



GRAPH NO: 6 COMPARISON OF WAIST HIP RATIO AMONG STUDY GROUPS

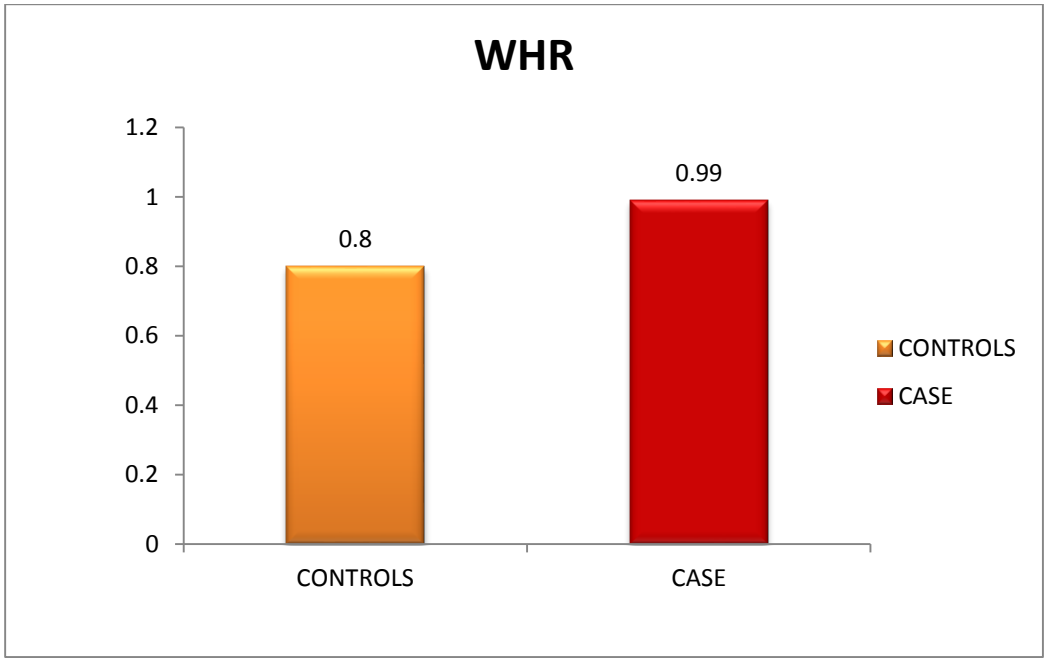


Table No: 2 compares the weight, body mass index, waist circumference, hip circumference and waist hip ratio of the controls and obese groups. The p value of all parameters were very highly significant.

TABLE NO: 3				
COMPARISON OF RESTING SYSTOLIC AND DIASTOLIC BLOOD PRESSURE AMONG STUDY GROUPS				
VARIABLE	STUDY GROUP	MEAN	SD	P-VALUE
RESTING SBP	CONTROLS	112	6.34	0.001**
	OBESE	116.72	7.57	
**** highly significant**				
RESTING DBP	CONTROLS	70.28	5.22	0.012*
	OBESE	73.24	6.26	
*** significant**				

Table No: 3 compares the resting SBP and the DBP among the study groups. The p value of the resting systolic blood pressure was highly significant and that of the resting diastolic blood pressure was also significant

Comparison of Resting HRV among the study group:

Comparison of time domain measures among the study groups:

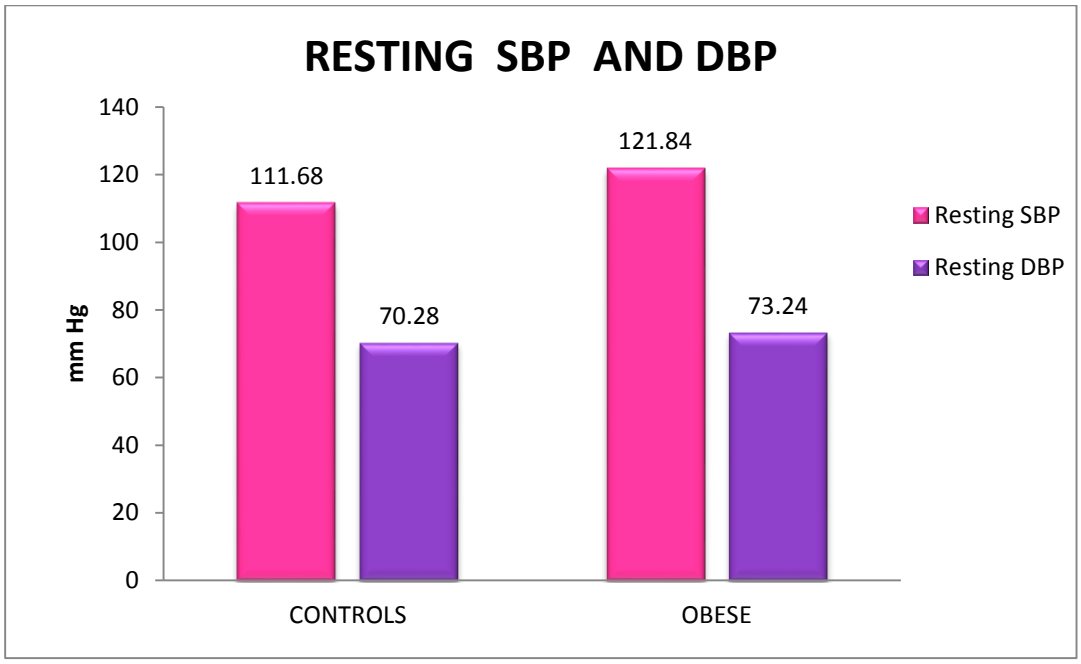
Table No: 4 compares the time domain measures namely SDNN and the Mean HR among the controls and the obese. The P value for SDNN was highly significant and that of the mean HR was very highly significant.

TABLE NO: 4				
COMPARISON OF SDNN AMONG STUDY GROUPS				
VARIABLE	STUDY GROUP	MEAN	SD	P-VALUE
SDNN	CONTROLS	60.14	15.50	0.002**
	OBESE	52.20	8.39	
**** highly significant**				
COMPARISON OF MEAN HEART RATE AMONG STUDY GROUPS				
MEAN HR	CONTROLS	74.30	2.79	0.000***
	OBESE	77.06	4.18	
***** very highly significant**				

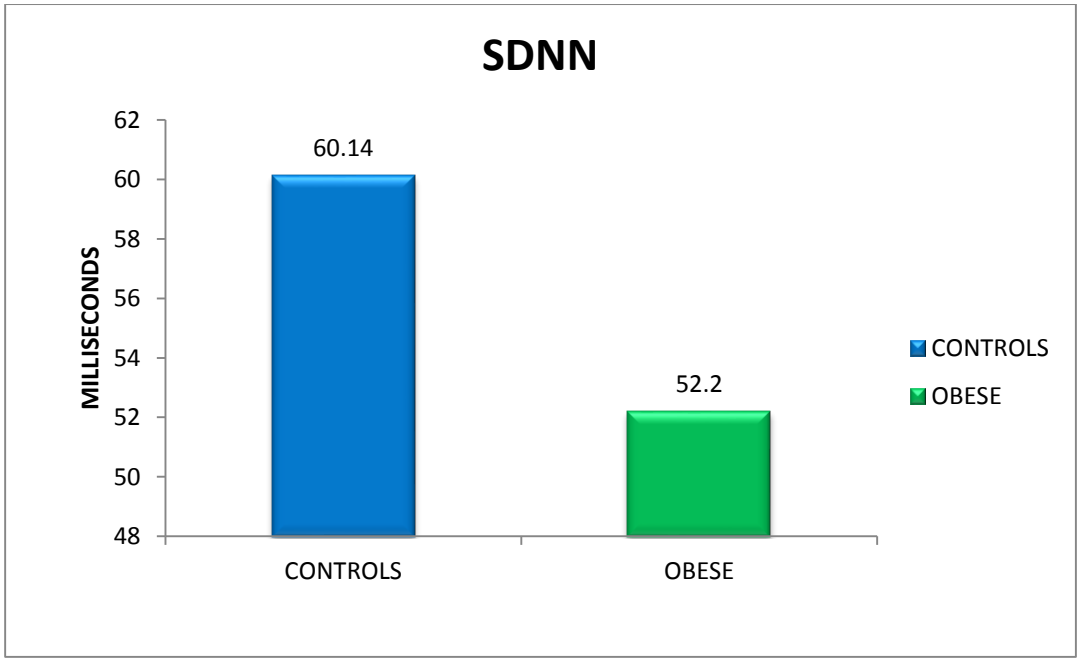
Comparison of Frequency domain measures among the study groups:

TABLE NO: 5				
VARIABLE	STUDY GROUP	MEAN	SD	P-VALUE
COMPARISON OF LF (nu) AMONG STUDY GROUPS				
LF nu	CONTROLS	40.47	9.58	0.000***
	OBESE	56.21	10.95	
COMPARISON OF HF (nu) AMONG STUDY GROUPS				
HF nu	CONTROLS	59.46	9.13	0.000***
	OBESE	43.86	11.12	
***** very highly significant**				
COMPARISON OF LF (nu)/ HF (nu) RATIO AMONG STUDY GROUPS				
LF/HF RATIO	CONTROLS	0.72	0.29	0.000***
	OBESE	1.42	0.59	
***** very highly significant**				

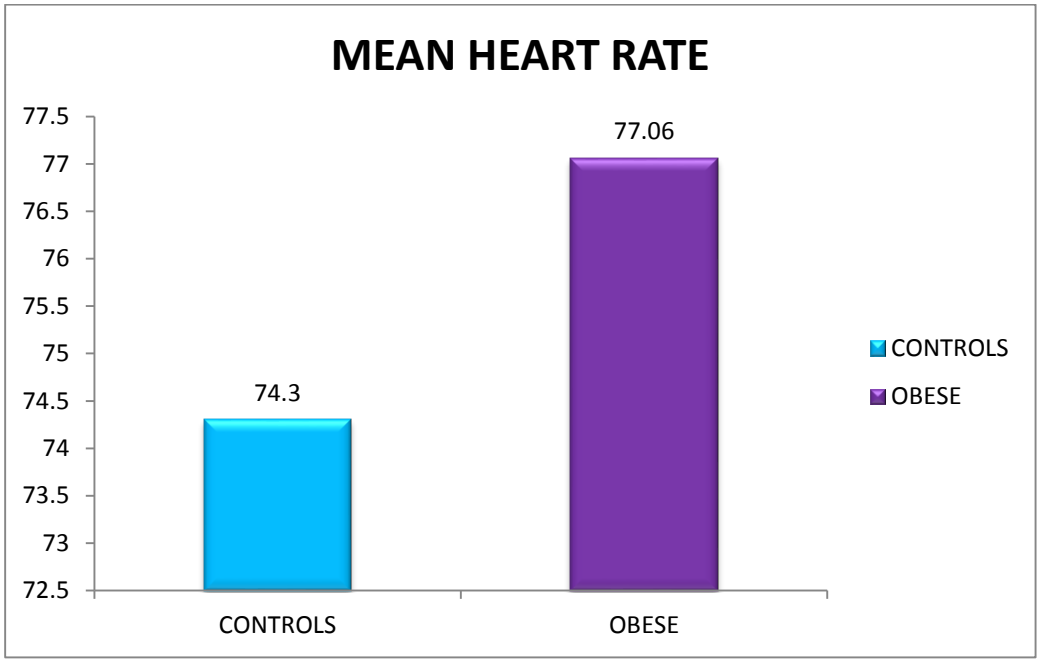
GRAPH NO: 7 COMPARISON OF RESTING SYSTOLIC AND DIASTOLIC BLOOD PRESSURE AMONG STUDY GROUPS



GRAPH NO : 8 COMPARISON OF SDNN AMONG STUDY GROUPS



GRAPH NO: 9 COMPARISON OF MEAN HEART RATE AMONG STUDY GROUPS



GRAPH NO: 10 COMPARISON OF LF nu AND HF nu AMONG STUDY GROUPS

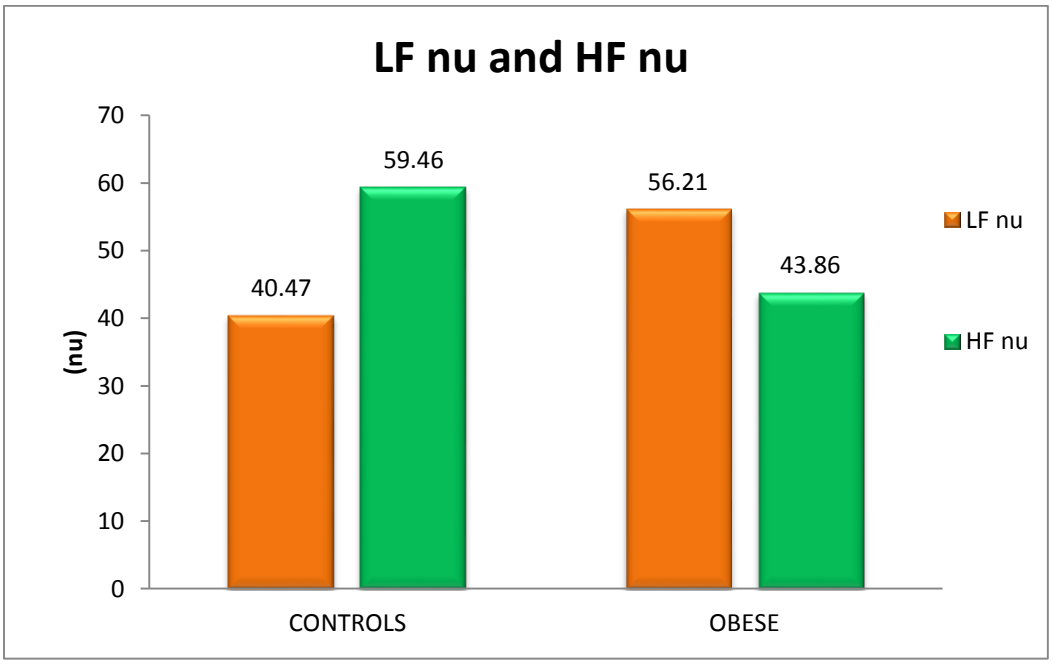
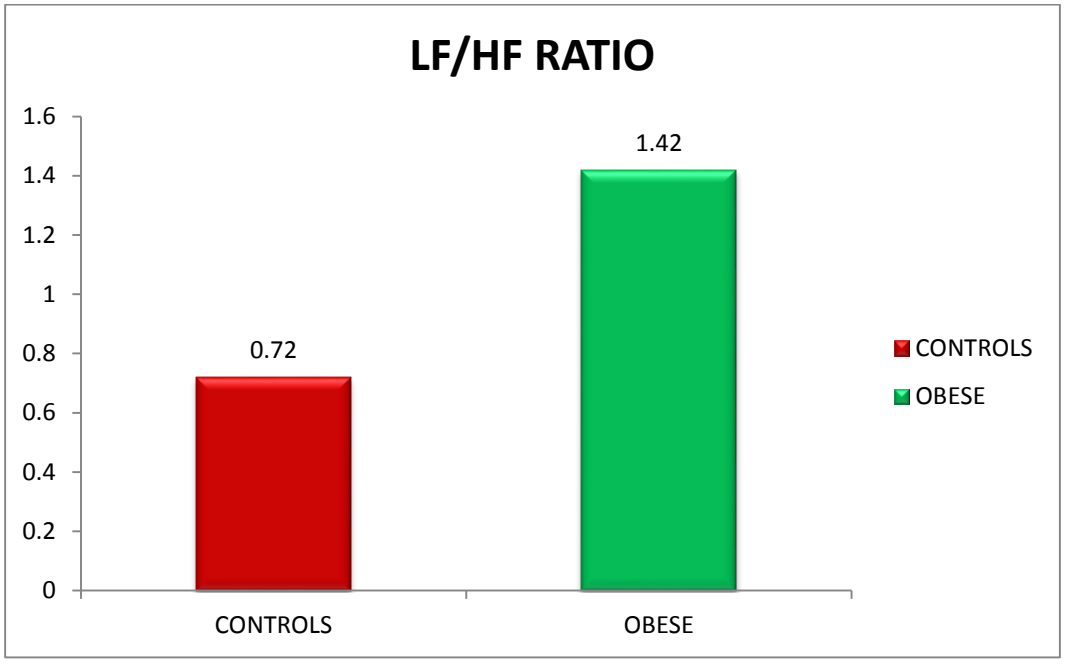


Table No: 5 compares the important Frequency Domain measures among the study groups. The P value of LF and HF were very highly significant. The P value of LF/HF ratio was also very highly significant.

Comparison of Autonomic Function Tests among study groups:

TABLE NO: 6				
COMPARISON OF ORTHOSTATIC STANDING TEST 30/15 RATIO AMONG STUDY GROUPS				
VARIABLE	STUDY GROUP	MEAN	SD	P-VALUE
30/15 RATIO	CONTROLS	1.16	0.04	0.011*
	OBESE	1.11	0.11	
*** significant**				
COMPARISON OF OS SYTOLIC BP AMONG STUDY GROUPS				
OST SBP	CONTROLS	-4.04	4.84	0.000***
	OBESE	2.96	6.65	
***** very highly significant**				
COMPARISON OF OS DIASTOLIC BP AMONG STUDY GROUPS				
OST DBP	CONTROLS	-1.04	2.56	0.000***
	OBESE	1.44	3.36	
***** very highly significant**				

GRAPH NO: 11 COMPARISON OF LF/HF RATIO AMONG STUDY GROUPS



GRAPH NO: 12 COMPARISON OF ORTHOSTATIC STANDING TEST 30/15 RATIO AMONG STUDY GROUPS

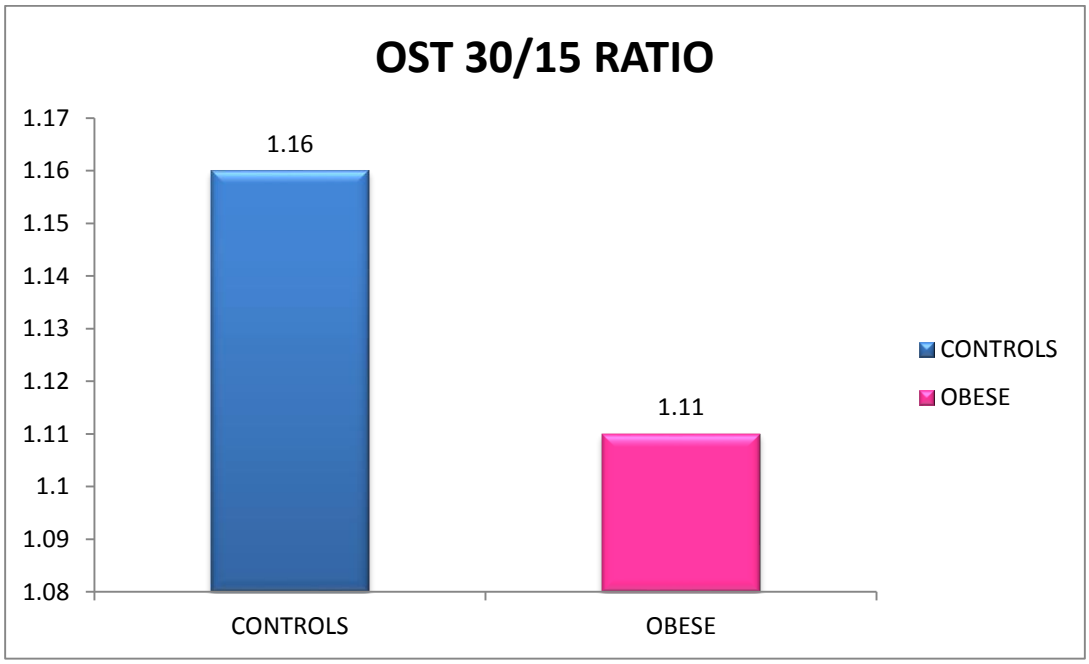


Table No: 6 compares the orthostatic standing test results among the study groups. Comparison of 30/15 ratio showed a statistically significant P value. The P value of orthostatic standing SBP and DBP at 1minute were very highly significant.

TABLE NO: 7

COMPARISON OF DEEP BREATHING TEST EXPIRATION/INSPIRATION (E/I) RATIO AMONG STUDY GROUPS

VARIABLE	STUDY GROUP	MEAN	SD	P-VALUE
E/I RATIO	CONTROLS	1.27	1.23	0.017*
	OBESE	0.09	0.09	

*** significant**

Table No: 7 compares the Expiration/Inspiration Ratio obtained by deep breathing test among study groups. The P value of E/I ratio was statistically significant.

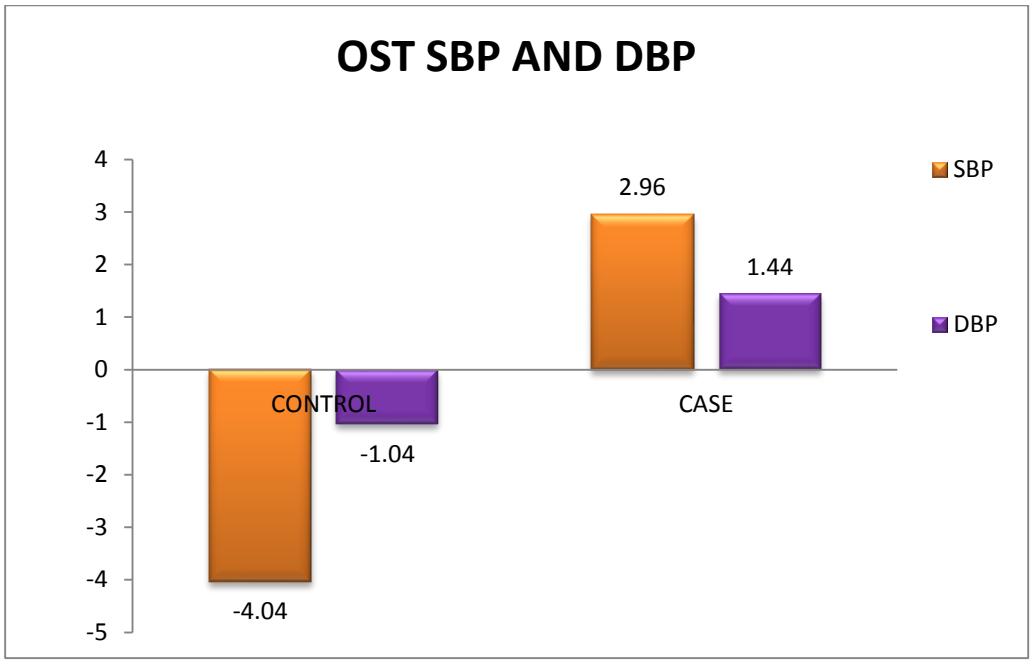
TABLE NO: 8

COMPARISON OF VALSALVA RATIO (VR) AMONG STUDY GROUPS

VARIABLE	STUDY GROUP	MEAN	SD	P-VALUE
VR	CONTROLS	1.37	0.16	0.001**
	OBESE	1.28	0.12	

**** highly significant**

GRAPH NO:13 COMPARISON OF ORTHOSTATIC STANDING SYSTOLIC AND DIASTOLIC BLOOD PRESSURE AMONG STUDY GROUPS



GRAPH NO: 14 COMPARISON OF E/I RATIO AND VALSALVA RATIO AMONG STUDY GROUPS

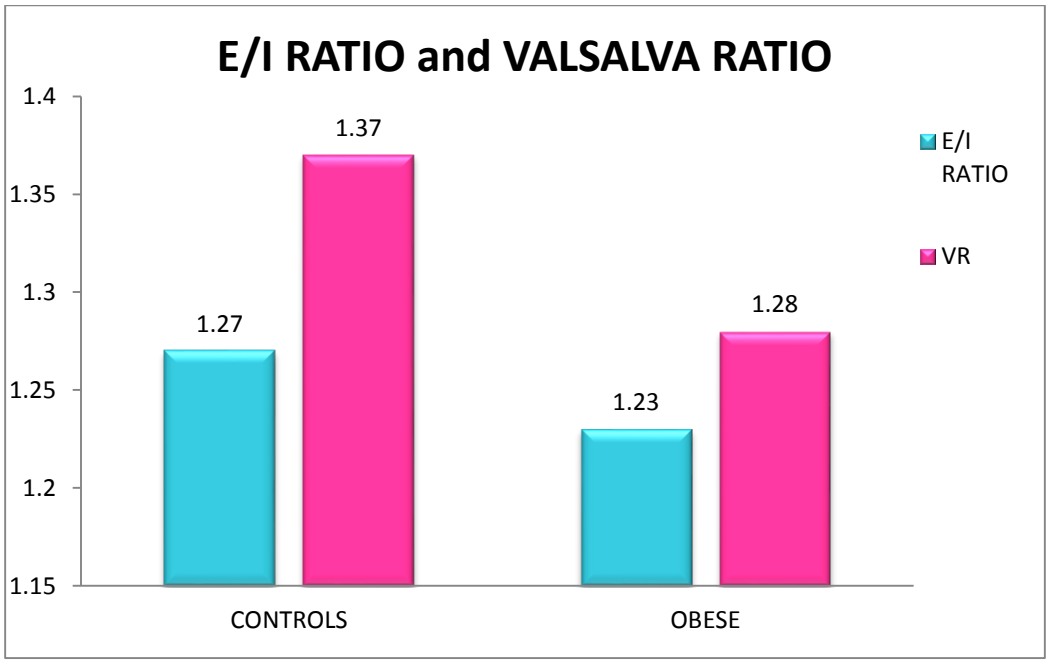


Table No: 8 Compares the mean and standard deviation of Valsalva Ratio (VR) among the study groups. The P value obtained was highly significant.

TABLE NO: 9				
COMPARISON OF ISOMETRIC HANDGRIP SYTOLIC AND DIASTOLIC BLOOD PRESSURE AMONG STUDY GROUPS				
VARIABLE	STUDY GROUP	MEAN	SD	P-VALUE
IHG SBP	CONTROLS	5.96	2.66	0.019*
	OBESE	7.24	2.68	
*** significant**				
IHG DBP	CONTROLS	12.08	3.33	0.004**
	OBESE	14.04	3.29	
**** highly significant**				
COMPARISON OF COLD PRESSOR TEST DIASTOLIC BLOOD PRESSURE AMONG STUDY GROUPS				
CPT DBP	CONTROLS	8.20	2.11	0.005**
	OBESE	9.76	3.17	
**** highly significant**				

Table No: 9 Compares the raise in SBP and DBP in Isometric handgrip test and raise in diastolic blood pressure in the Cold pressor test among the controls and the obese groups. The P value of the isometric handgrip systolic BP was statistically

significant while the isometric handgrip diastolic BP was highly significant and the Cold pressor test diastolic BP was also highly significant.

TABLE NO: 10

COMPARISON OF SERUM LEPTIN LEVELS AMONG STUDY GROUPS

VARIABLE	STUDY GROUP	MEAN	SD	P-VALUE
S.LEPTIN	CONTROLS	6.92	4.62	0.000
	OBESE	24.28	7.63	

***** very highly significant**

Table No: 10 compares the serum leptin levels among the study groups. The P value was very highly significant.

TABLE NO: 11

CORRELATION OF SERUM LEPTIN LEVELS

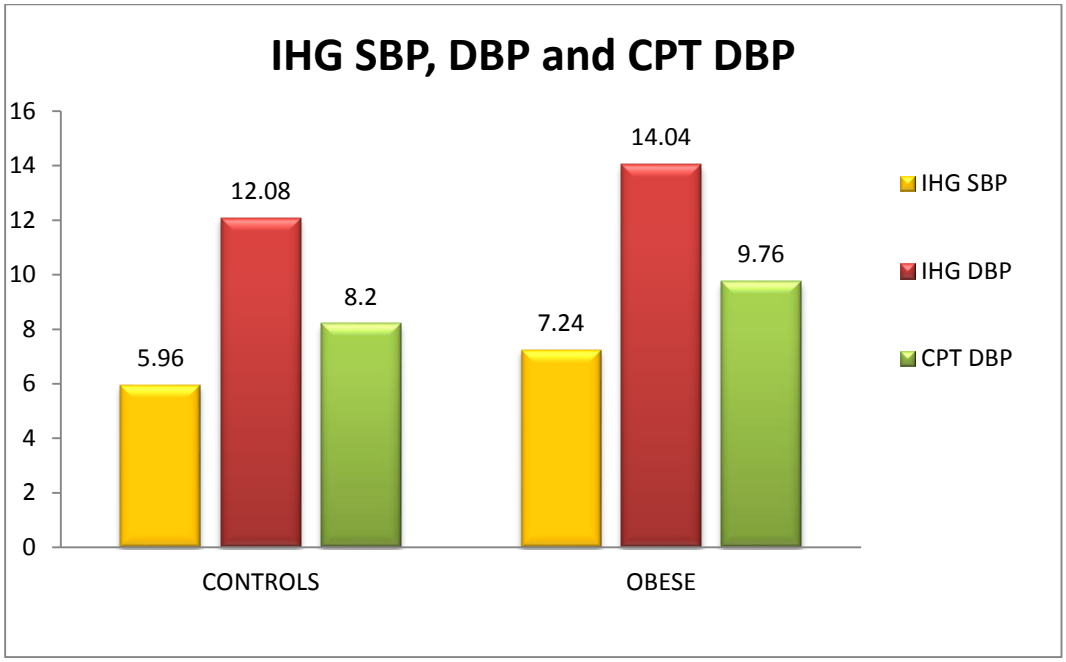
WITH OBESITY AND HRV INDICES

S.LEPTIN	BMI	WC	LF nu	HF nu	LF/HF
'r'	0.62	0.42	0.52	-0.50	0.51

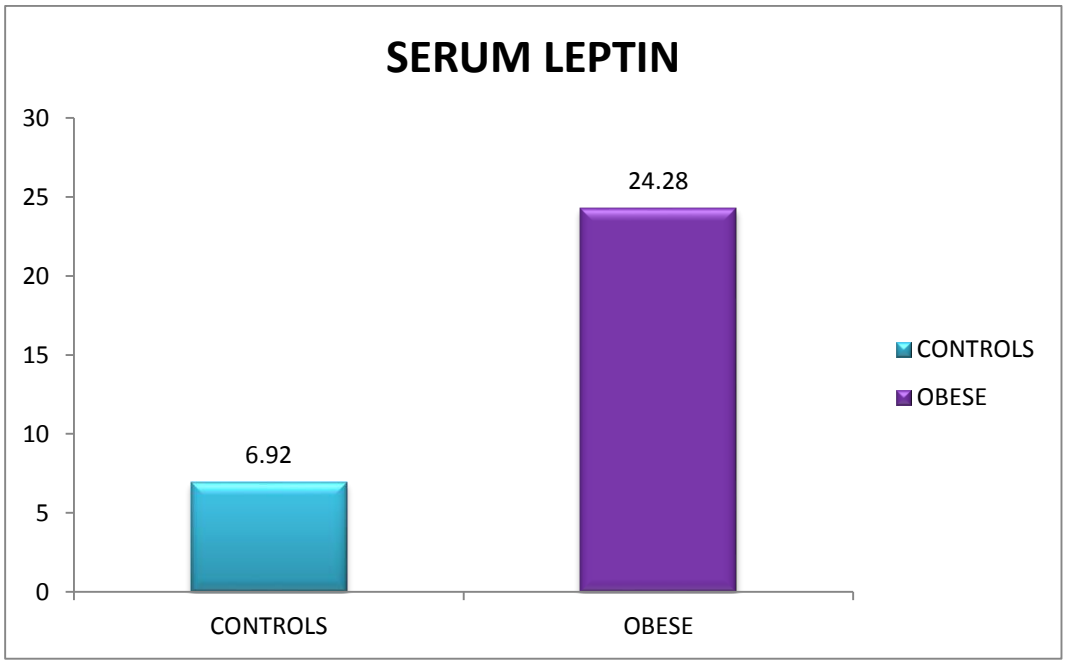
'r' is Pearson's correlation co efficient

Table No: 11 shows the correlation of serum leptin levels in obese individuals positively correlated with BMI and WC. It also shows the correlation of serum

GRAPH NO: 15 COMPARISON OF ISOMETRIC HAND GRIP SYSTOLIC, DIASTOLIC BP AND COLD PRESSOR TEST DIASTOLIC BP AMONG STUDY GROUPS



GRAPH NO: 16 COMPARISON OF SERUM LEPTIN LEVELS AMONG STUDY GROUPS



leptin with HRV indices LF, HF, LF/HF ratio. Leptin is positively correlated with LF and LF/HF ratio while it shows negative correlation with HF.

TABLE NO: 12		
CORRELATION OF HRV INDEX (LF/HF) WITH BMI AND WC		
HRV- LF/HF RATIO	BMI	WC
'r'	0.59	0.57
'r' is Pearson's correlation co efficient		

Table No: 12 shows the correlation of HRV index LF/HF ratio in obese group with BMI and WC both being positively correlation.

DISCUSSION

6. DISCUSSION

The purpose of the present study was to evaluate the cardiovascular autonomic functions and assess the serum leptin levels in young obese individuals. The study was aimed at including fifty young obese individuals of the age 19 to 25 years with BMI $\geq$ 25 of both sexes and fifty age matched normal controls with BMI of 18.5 -24.9.

There was a marked difference in the weight of the controls and the cases. The P value was 0.000 which was very highly significant. The BMI of the controls was 20.48± 1.09 and that of the obese was 31.50± 2.25. The P value was less than 0.0001, very highly significant.

The waist circumference of the obese group was increased significantly when compared to the controls. There was also a significant increase in the Hip Circumference of the obese. The WHR of the controls was 0.80 ± 0.04 and that of the young obese was 0.99 ± 0.02. The p value was 0.000 which was very highly significant.

Rajalakshmi et al has shown similar differences in physical characters among the controls and obese in her study. Waist circumference and waist to hip ratio are direct indicators of abdominal obesity that is visceral fat. A greater waist to hip ratio and increased waist circumference were independently associated with a significantly increased risk of coronary heart disease (Yusuf S et al, 2004)[108]

Resting Blood Pressure:

The resting systolic blood pressure in the controls was 112 ± 6.34 while the resting systolic blood pressure in the obese group was 116.72± 7.57, which was significantly higher with a p value of 0.001. The resting diastolic blood pressure of controls was 70.28 ± 5.22 where as it was significantly higher that is 73.28 ± 6.26 in the obese group with a statistically significant p value of 0.012.

Both the systolic and the diastolic blood pressure were within normal range but with a significant difference among the study groups. Those who were known hypertensive were not included in this study as hypertensive subject show altered ANS functions as stated by Julius et al.

This increase in blood pressure in obesity could be attributed to direct effect of obesity on hemodynamics such as increase in blood volume, stroke volume and cardiac output and mechanisms linking obesity to increase in peripheral vascular resistance such as endothelial dysfunction and effect of cytokines (Bjorntop et al, 1990)[109]. The risk of hypertension is increased in obesity (Stamler R et al)[110].

The study was accomplished using resting HRV and a battery of cardiovascular autonomic function tests and assessment of serum leptin was done. The integrity of sympathetic and parasympathetic components of the autonomic nervous system and the sympathovagal balance were assessed and thereby

substantiate the role of autonomic dysfunction and leptin in obesity and its comorbidities.

The cardiovascular autonomic functions are strongly influenced by sympathetic and parasympathetic divisions. The sympathetic nervous system has the control on the myocardial contractility and heart rate where as the parasympathetic effect is essentially on the heart rate.

Time Domain Measures of Resting HRV:

The SDNN in the control group was 60.14 ± 15.50 and that in the obese was 52.20 ± 8.39. The difference in the value shows that there is a significant decrease of standard deviation of normal to normal interval (SDNN) in the obese group with a p value of 0.002.Chethan et al has shown similar results in his study.

Similar results were shown by Emdin M et al, 2001[111]. Similar findings of reduced SDNN in obese have been shown by Archana et al, 2013[112]. A reduction in SDNN, a parameter which reflects parasympathetic activity in this study implies a reduction in parasympathetic activity in obese.

The Mean HR in the control group was 74.30 ± 2.79 while the mean heart rate in the obese group was raised to 77.06 ± 4.18 with a very highly significant p value of 0.000. Though the mean HR in both the groups are within normal limits

there is a significant increase in mean HR in the obese group which could be attributed to parasympathetic withdrawal in the obese

Rajalakshmi et al has got similar results in her study. This could be due to hemodynamic changes in obesity. An increase in body weight is associated with an increase in mean heart rate due to decline in parasympathetic tone as stated by Hirsch J et al in his study[113]. Palatini et al has stated in his study that higher heart rate could be a marker of relative sympathetic dominance and is an independent marker of mortality in various conditions.

Though the time domain parameters yields better results in 24 hour Holter monitoring than short term resting HRV monitoring which was used in this study as stated by Saul JP et al, a significant decrease in SDNN and increase in mean HR indicates parasympathetic withdrawal.

Frequency Domain Measures of Resting HRV:

This study shows a highly significant variation in frequency domain variables. The LF and HF values are altered by the total power changes. To minimize this effect normalized values are taken. The Low Frequency in normalized units in the controls was 40.47 ± 9.58 and in the obese was 56.21±10.95, with very highly significant increase in the obese with a P value of 0.000 which indicates an increase in the sympathetic activity in the obese group.

The HF nu in the controls was 59.46 ± 9.13 and in the obese was 43.86 ± 11.12 which shows a very highly significant decrease in the obese group with a P value of 0.000 which implies parasympathetic withdrawal as HF nu is an indicator of vagal activity.

The LF/HF ratio which is an accurate measure of sympathovagal balance has increased in the obese group when compared to the control group with a very highly significant P value of 0.000, which implies overall sympathovagal imbalance in the obese with sympathetic over activity and parasympathetic withdrawal. These results were consistent with the results of the studies of Rajalakshmi et al, Chethan et al and Archana et al. Mehmet Erkan Altuncu et al, Karason k et al and Chen- chu fu et al in their studies have shown similar results.

Orthostatic Standing Test:

The cardiovascular reflex changes on active standing after a period of rest in supine posture provides information on the integrity the baroreflex pathway. There occurs pooling of blood in the lower extremities on standing due to gravity which causes a reduced cardiac output and an immediate fall in BP. This is accompanied by an increase in heart rate which is recorded as a maximum decrease in RR interval, a maximum at about the 15th beat. Contraction of the limb and abdominal vessels on active standing causes an increased venous return and a rise in BP which results in the slowing of heart rate which is recorded as an increase in RR interval a maximum at about the 30^{th} beat (Borst et al).

Thus the ratio of the maximum RR interval and the minimum interval which is the 30/15 ratio is a measure of parasympathetic activity. The blood pressure response to active standing is a function of sympathetic system.

In this study, the orthostatic standing test 30/15 ratio was decreased in the obese group (1.11±0.11) when compared to the control group (1.16±0.04) with significant P value of 0.011, which indicates a decreased parasympathetic activity in the obese.

After 1-3 minutes of standing, the systolic blood pressure of the controls showed a fall of 4.04± 4.84 mm Hg while that of the obese group showed an increase of 2.96 ± 6.65 mm Hg with a significant P value of 0.000 and the diastolic blood pressure of the control group showed a fall of 1.04±2.56 mm Hg while that of the obese group showed an increase in diastolic blood pressure by 1.44±3.36 with a significant P value of 0.000. These findings substantiate sympathetic over activity in the obese group.

Deep breathing test:

The principle behind this test is sinus arrhythmia. During inspiration the heart rate increases and the reverse occurs in expiration. The ratio between the longest RR interval during expiration and the shortest interval during inspiration (E/I ratio) are calculated and compared between the study groups.

The E/I ratio in the obese group (0.09 ± 0.09) was significantly reduced when compared with the control group (1.27 ± 1.23) with a P value of 0.017. This

result was consistent with results shown by Chethan et al in his study. The decrease in E/I ratio in obese individuals suggests a decreased vagal action that parasympathetic withdrawal.

Valsalva maneuver:

The Valsalva ratio is calculated by the ratio of the longest RR interval after the strain (phase IV) and the shortest RR interval during the strain (phase II). It is a measure of parasympathetic function. To measure blood pressure accurately in the four phases of the maneuver is difficult manually by sphygmomanometer, which requires a beat to beat blood pressure monitor.

VR in the obese (1.28 ± 0.12) was significantly lower as compared to the controls (1.37 ± 0.16) with p value of 0.001 which implies decrease in parasympathetic activity. Valsalva ratio ≥ 1.21 is considered as normal in younger age group (Ewing et al).

Isometric handgrip test:

Sustained isometric hand grip against resistance causes an increase in heart rate and arterial blood pressure. This cardiovascular reflex is sympathetically mediated and one of the simple non invasive measures of sympathetic reactivity of autonomic nervous system that reflects the activity of peripheral sympathetic system (Bannister et al). Thus, the normal response is increase in the diastolic

blood pressure and heart rate after the maximum voluntary contraction was continued for one minute.

In this study, the systolic blood pressure in the obese was higher than that of the controls with a significant P value of 0.019. The diastolic blood pressure of the obese was 14.04 ± 3.29 which was higher than that of the controls (12.08 ±3.33) with a significant p value 0.004. This result substantiates that there is an increased sympathetic activity in the obese group.

Cold pressor test:

The cold pressor test helps in assessing the sympathetic nervous system. The normal response to immersion of a hand in ice water involves reflex arterial vasoconstriction producing increase in blood pressure and cardiac output by activation of afferent temperature, pain receptors in the skin. The sympathetic activation causes increase in diastolic blood pressure.

The diastolic blood pressure of the control group was 8.20 ± 2.11and that of the obese group was 9.76 ± 3.17 with a significant P value 0.004. The increase in the diastolic blood pressure in the obese substantiates sympathetic over activity.

Serum Leptin levels:

On assessing by ELISA method, we could find that the serum leptin level in the control group (6.92 ± 4.62) and that of the obese group (24.28 ± 7.63) showed

a great difference. The obese group showed a very significant high level of serum leptin with a P value of 0.00. Many of the early studies have shown similar results.

Masoud Y Al Maskariet al and Adel A Alnaqdy et al have shown similar findings in their study. This present study result is consistent with hyperleptinemia in obesity. Leptin receptor tolerance could be a cause for such an elevated level of leptin in obesity. Its level also positively correlated with BMI and sympathetic HRV parameter LF and may be associated with the cardiovascular risks in obesity.

Correlation of serum leptin levels with HRV and obesity indices:

The serum leptin levels of the young obese individuals had a positive correlation with the obesity indices namely body mass index and waist circumference. Thus an increase in BMI and waist circumference may be associated with elevated leptin levels and hence an increased cardiovascular risk.

The leptin levels in obese also had a positive correlation with LF nu which is an indicator of sympathetic parameter of HRV, negative correlation with the HF nu, an indicator of parasympathetic activity, and a positive correlation with LF/HF ratio the marker of sympathovagal balance. This result suggests that a raise in leptin level is prone to cause a raise in sympathetic hyperactivity leading on to autonomic dysfunction.

Correlation of HRV index (LF/HF) with BMI and Waist Circumference:

LF/HF ratio, the major indicator of sympathovagal balance shows a positive correlation with the body mass index and waist circumference in young obese group and hence an increase in body mass index and waist circumference as in obesity is associated with autonomic dysfunction.

Thus the evaluation of all these findings from all the autonomic function tests done suggest autonomic dysfunction in young obese individuals with parasympathetic withdrawal and sympathetic predominance, and associated with an elevated level of serum leptin which may have a role in the etiopathogenesis of obesity and its comorbidities with increased cardiovascular risks.

Limitations of the study:

A prospective study with a follow up after weight reduction would be preferred. It would have been better if Catecholamine hormonal assay that is a direct measure of sympathetic system were also done.

CONCLUSION

7. CONCLUSION

Autonomic Function tests in young obese individuals indicate that there is a definite sympathovagal imbalance in the form of sympathetic overactivity and parasympathetic withdrawal. Chronic activation of the sympathetic nervous system makes them more prone for adverse cardiovascular events at an early age.

In young obese individuals, Serum leptin levels are significantly higher correlating with BMI and waist circumference thus playing a role in etiopathogenesis of obesity and in increasing the cardiovascular risks and other co morbidities. Serum leptin levels correlated well with HRV parameters substantiating its role in stimulating the sympathetic nervous system.

The battery of autonomic function tests is an effective tool in identifying the autonomic dysfunction at an earlier stage. Understanding the role of the sympathetic nervous system and the serum leptin levels in obesity might help in the treatment of obesity and prevent further complications in this disease. Further studies with hormonal assays would warrant a better understanding of the etiopathogenesis of obesity and other associated metabolic complications

SUMMARY

8. SUMMARY

Obesity is a common nutritional health problem associated with metabolic and hemodynamic changes that lead to increased cardiovascular risks affecting a wide spectrum of age groups. We aimed at evaluating the cardiovascular autonomic functions and serum leptin levels in normal young obese individuals.

50 young obese with BMI $\geq$ 25, of both sexes aged 18 to 25 years and 50 age matched normal individuals of both sexes with BMI 18.5 to 24.9 were subjected to a battery of autonomic function tests including resting HRV and their serum leptin levels were assessed using ELISA method.

On analyzing the data obtained from the study, we could find that young obese individuals had autonomic dysfunction with sympathetic predominance and parasympathetic withdrawal, and their serum leptin level was elevated and correlated well with BMI. Sympathovagal imbalance with parasympathetic inhibition and sympathetic overactivity and elevated levels of leptin plays a vital task in etiopathogenesis of obesity and its comorbidities especially the increased cardiovascular risks.

Early diagnosis of autonomic dysfunction using the non invasive autonomic function tests could help in the prevention of adverse cardiac events by early intervention by life style modification with alterations in the dietary pattern and physical activities so as to reduce weight.

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ANNEXURES

INSTITUTE ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg. No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 044 25363970

CERTIFICATE OF APPROVAL

To

Dr.V.Sumathi.
Post Graduate M.D.(Physiology)
Madras Medical College, Chennai -3.

Dear Dr.V.Sumathi.

A study of Cardiovascular Autonomic functions and Serum leptin levels in young obese individuals " No.20102014.

1. Dr.C. Rajendran,MD	: Chairperson
2. Dr.V.Vimala,M.D. Dean,MMC,Chennai-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi,M.D.,Vice-Principal,MMC,Ch-3	: Member Secretary
4. Prof.R.Nandhini,MD.Inst.of Pharmacology, MMC	: Member
5. Dr. Raghumani, Director ic.Institute of Surgery	: Member
6. Prof. Ramadevi,Director i/c. Bio Chemistry MMC	: Member
7. Prof.Saraswathy,MD.Director,Pathology, MMC	: Member
8. Prof.S.G.Sivachidambaram,MD. Director i/c. Institute Internal Medicine, MMC,	: Member
9. Thiru S.Rameshkumar, Administrative Officer	: Lay Person
10.Thiru S.Govindasamy,BA.BL.	: Lawyer
11.Tmt.Arnold Sauline,M.A.MSW	: Social Scientist

We approve the proposal to be conducted in its presented in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

INFORMED CONSENT FORM

Title of the study "A study of Cardiovascular Autonomic Functions and Serum Leptin Levels in Young Obese individuals"

Name of the Participant:

Name of the Principal Investigator: Dr.V.Sumathi

Name of the Institution:

Institute of Physiology and Experimental Medicine,
Madras Medical College and Govt. General Hospital,
Chennai - 3

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in

"**A study of Cardiovascular Autonomic Functions and Serum Leptin Levels in Young Obese individuals**"

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I have been advised about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past _____month(s).
9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.

10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.
12. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
13. I have understand that my identity will be kept confidential if my data are publicly presented.
14. I have had my questions answered to my satisfaction.
15. I have decided to be in the research study.
I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____

Date_____

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு : உடல் பருமன் உள்ளவர்களிடம் அட்டானாமிக் நரம்பு மண்டலத்தில் ஏற்படும் மாற்றங்களை, இருதய துடிப்பு வேறுபடுதல் மூலமாக கண்டறிதல் மற்றும் °£ரம் ¦Ä்Ê்ன் அளவை ஆராய்ந்து அறிதல்

ஆராய்ச்சியாளர் பெயர்: மரு. வே.சுமதி

ஆராய்ச்சி நடக்கும் இடம்: சென்னை மருத்துவக் கல்லூரி

பெயர்:

வயது:

பாலினம்: ஆண்/பெண்

பங்கு பெறுபவர் அடையாள எண்:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கமும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.

எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்து கொண்டு நான் எனது சம்மதத்தை தெரிவிக்கிறேன்.

எனது அட்டானாமிக் நரம்பு மண்டலத்தில் ஏற்படும் மாற்றங்களை, இருதய துடிப்பு வேறுபடுதல் மூலமாக கண்டறிதல் மற்றும் °£ரம் ¦Ä்Ê்ன் அளவை பரிசோதனை செய்ய முழு சம்மதம்.

இந்த ஆராய்ச்சியில் யாருடைய நிர்பந்தமுமின்றி சொந்த விருப்பத்தின் பேரில் சம்மதிக்கிறேன்.

இந்த ஆராய்ச்சியில் இருந்து நான் எந்த நேரமும் பின் வாங்கலாம் என்றும், அதனால் எந்த பாதிப்பும் எற்படாது என்பதையும் புரிந்து கொண்டேன்.

நான் உடல் பருமன் குறித்த இந்த ஆராய்ச்சியின் விவரங்கள் கொண்ட தகவல்களை பெற்றுக்கொண்டேன்.

°£ரம் ¦Ä்Ê்ன் அளவை பரிசோதனைக்கு ஊசி மூலம் இரத்தம் எடுக்க சம்மதிக்கிறேன். இரத்தம் எடுக்கும் போது வலி, அரிப்பு, மயக்கம், போன்ற பின் விளைவுகள் எற்படலாம் என்று தெரிந்து கொண்டேன்.

நான் என்னுடைய சுய நினைவுடன் மற்றும் முழு சம்மதத்துடன் இந்த ஆராய்ச்சிக்கு என்னை பரிசோதிக்க சம்மதிக்கிறேன்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

நாள்:

இடம் :

PROFORMA

Name:

Age:

Sex:

Address:

Occupation:

Complaints/duration:

History of present illness:

Past history:

Personal history:

History of any drug intake:

History of associated illness:

a. Diabetes
b. Hypertension
c. Ischemic heart disease
d. Respiratory diseases
e. Renal diseases

Investigations :

Fasting & post prandial blood Sugar

Serum electrolytes

Lipid profile

Thyroid profile

S.Testosterone

General examination:

Temperature:

Pulse rate:

Blood pressure:

Height:

Weight:

BMI: wt in kg / Ht^2 in m^2

Waist hip ratio:

Systemic examination:

Cardiovascular system:

Respiratory system:

Gastrointestinal system:

Central nervous system:

MASTER CHART CONTROLS

S.NO	AGE (YRS)	SEX (M/F)	Ht (m)	Wt (Kg)	BMI (kg/m^2)	WAIST (cms)	HIP (cms)	WHR	SBP (mm Hg)	DBP (mm Hg)	Mean HR (/min)	SDNN (msec)	LF nu	HF nu	LF/HF	OST 30/15 ratio	OST SBP_1	OST DBP_1	E/I ratio	VR	IHG SBP	IHG DBP	CPT DBP	S.Leptin (ng/ml)
1	19	F	1.62	50	19.05	74	92	0.80	114	70	72	43	46.8	54.2	0.86	1.15	-8	-2	1.42	1.34	6	12	6	4
2	21	M	1.71	58	19.84	76	88	0.86	110	68	70	68	43.3	56.7	0.76	1.12	-4	-4	1.22	1.21	4	10	8	2
3	18	F	1.68	54	19.13	72	94	0.77	118	72	74	83	55.6	44.4	1.25	1.08	-2	0	1.32	1.38	8	14	10	1
4	19	M	1.7	55	19.03	73	86	0.85	98	60	70	65	33.7	66.3	0.51	1.16	-6	-2	1.28	1.24	6	10	6	3
5	22	M	1.55	52	21.64	75	87	0.86	110	60	72	53	45.8	54.2	0.85	1.09	-2	0	1.18	1.54	2	8	8	2
6	21	M	1.58	52	20.83	77	89	0.87	110	70	71	37	48.5	51.7	0.94	1.19	4	0	1.24	1.29	4	14	10	8
7	20	F	1.68	54	19.13	76	92	0.83	112	70	74	76	32.7	66.3	0.49	1.18	-8	-6	1.2	1.43	10	16	6	9
8	19	F	1.67	54	19.36	75	94	0.80	106	66	73	64	52.7	47.3	1.11	1.13	-4	-2	1.22	1.25	8	10	8	12
9	20	M	1.57	53	21.50	76	90	0.84	110	70	72	52	47.8	55.2	0.87	1.21	-6	-4	1.18	1.63	4	8	8	7
10	21	M	1.62	55	20.96	74	87	0.85	120	78	70	66	37.9	63.1	0.60	1.23	-2	0	1.3	1.32	6	12	10	11
11	21	F	1.63	54	20.32	75	89	0.84	108	76	74	94	43.4	56.6	0.77	1.12	4	2	1.26	1.29	4	10	8	8
12	20	M	1.61	56	21.60	76	88	0.86	110	72	76	51	45.4	54.6	0.83	1.08	-10	-4	1.24	1.22	4	10	12	1
13	19	F	1.55	52	21.64	78	96	0.81	100	60	74	49	56.4	43.6	1.29	1.15	8	4	1.22	1.27	2	8	10	10
14	19	F	1.65	52	19.10	75	94	0.80	108	70	72	89	39.3	60.7	0.65	1.19	-2	0	1.21	1.62	10	18	8	13
15	18	F	1.56	50	20.55	74	92	0.80	110	70	74	91	34.9	65.1	0.54	1.16	-6	0	1.44	1.18	4	10	6	9
16	21	M	1.54	51	21.50	74	86	0.86	112	72	78	48	49.5	50.5	0.98	1.18	-8	-4	1.24	1.21	2	8	8	6
17	21	F	1.6	51	19.92	75	90	0.83	106	68	74	50	48.6	58.4	0.83	1.13	-4	-2	1.34	1.33	4	10	8	11
18	22	M	1.55	50	20.81	76	94	0.81	110	72	72	56	22.8	77.2	0.30	1.21	-6	0	1.38	1.26	8	14	8	4
19	20	M	1.62	52	19.81	74	96	0.77	110	74	76	64	53.2	46.8	1.14	1.09	6	2	1.22	1.36	12	16	10	6
20	21	M	1.69	54	18.91	75	95	0.79	120	70	75	51	26.7	73.3	0.36	1.14	-6	0	1.24	1.24	6	14	6	7
21	19	F	1.62	50	19.05	76	97	0.78	108	62	74	48	40.9	59.1	0.69	1.11	8	4	1.23	1.65	2	12	8	14
22	18	M	1.68	54	19.13	74	89	0.83	118	74	73	37	43.8	56.7	0.77	1.08	-6	0	1.28	1.32	4	8	6	6
23	21	F	1.6	54	21.09	74	88	0.84	120	74	72	38	31.6	68.4	0.46	1.12	-10	4	1.36	1.21	8	16	4	10
24	21	F	1.57	52	21.10	73	87	0.84	122	72	78	53	50.8	49.2	1.03	1.1	-8	-4	1.26	1.66	12	18	8	9
25	20	F	1.54	52	21.93	75	92	0.82	118	74	74	36	59.3	40.7	1.46	1.14	-8	-2	1.17	1.28	6	14	12	17

MASTER CHART CONTROLS

S.NO	AGE (YRS)	SEX (M/F)	Ht (m)	Wt (Kg)	BMI (kg/m^2)	WAIST (cms)	HIP (cms)	WHR	SBP (mm Hg)	DBP (mm Hg)	Mean HR (/min)	SDNN (msec)	LF nu	HF nu	LF/HF	OST 30/15 ratio	OST SBP_1	OST DBP_1	E/I ratio	VR	IHG SBP	IHG DBP	CPT DBP	S.Leptin (ng/ml)
26	20	M	1.55	51	21.23	76	97	0.78	110	70	75	68	26.3	73.7	0.36	1.08	-4	-4	1.28	1.52	10	18	10	2
27	21	F	1.59	50	19.78	77	97	0.79	108	68	80	39	44.5	54.5	0.82	1.18	-6	0	1.26	1.22	4	8	8	3
28	20	M	1.6	52	20.31	76	96	0.79	110	64	78	57	29.8	67.2	0.44	1.16	-2	2	1.32	1.24	6	12	8	6
29	19	F	1.58	50	20.03	74	95	0.78	118	72	72	49	42.4	57.6	0.74	1.22	-6	-4	1.46	1.36	4	8	10	14
30	21	M	1.57	49	19.88	75	98	0.77	120	74	74	64	36	64	0.56	1.19	8	4	1.26	1.31	6	14	8	9
31	19	M	1.45	47	22.35	80	97	0.82	104	66	73	74	23.7	64.3	0.37	1.16	-4	0	1.38	1.42	8	16	6	2
32	22	M	1.53	48	20.50	77	95	0.81	114	72	75	57	40.2	59.8	0.67	1.18	-6	-2	1.16	1.38	8	14	10	7
33	21	F	1.48	48	21.91	79	96	0.82	110	70	80	76	25.7	74.3	0.35	1.22	8	4	1.24	1.26	10	16	8	19
34	20	M	1.78	59	18.62	78	97	0.80	112	68	79	69	30.4	69.6	0.44	1.16	-6	0	1.42	1.21	6	10	4	2
35	21	F	1.58	48	19.23	75	98	0.77	98	60	74	73	26.1	73.9	0.35	1.18	-8	-2	1.24	1.54	8	14	6	3
36	20	M	1.59	49	19.38	76	98	0.78	110	70	72	77	42.7	57.3	0.75	1.16	-4	0	1.16	1.62	6	18	8	1
37	20	F	1.68	55	19.49	78	99	0.79	120	80	73	83	32.7	67.3	0.49	1.14	-8	-4	1.42	1.24	4	8	6	12
38	19	M	1.58	51	20.43	78	97	0.80	116	74	78	52	43.4	56.6	0.77	1.19	-8	-2	1.23	1.32	4	10	6	4
39	20	M	1.57	52	21.10	77	96	0.80	114	70	75	61	38.5	61.5	0.63	1.15	-4	0	1.22	1.19	2	8	8	3
40	21	F	1.55	53	22.06	74	95	0.78	120	76	78	55	56.6	43.4	1.30	1.21	-4	-4	1.14	1.23	6	18	14	9
41	20	F	1.53	51	21.79	73	94	0.78	116	62	79	51	45.4	54.6	0.83	1.08	-6	0	1.26	1.72	8	14	8	1
42	20	F	1.65	53	19.47	74	96	0.77	100	64	72	90	34.9	65.1	0.54	1.14	-6	-2	1.16	1.27	10	16	12	10
43	19	M	1.7	55	19.03	70	96	0.73	108	68	70	50	38.4	61.6	0.62	1.19	-4	0	1.24	1.34	4	10	10	2
44	21	M	1.54	53	22.35	72	97	0.74	124	82	73	46	41.8	58.2	0.72	1.22	-8	-4	1.32	1.61	6	8	6	3
45	18	F	1.55	52	21.64	76	98	0.78	110	74	73	61	38.5	61.5	0.63	1.16	-6	-4	1.48	1.48	4	10	8	8
46	20	F	1.63	56	21.08	78	99	0.79	104	68	74	42	31.6	68.4	0.46	1.19	-4	-2	1.25	1.52	8	14	12	4
47	21	M	1.6	55	21.48	76	96	0.79	118	70	80	82	55.6	44.4	1.25	1.18	-10	0	1.23	1.26	2	10	8	1
48	20	F	1.59	54	21.36	73	98	0.74	120	80	78	46	29.6	71.4	0.41	1.15	-6	-4	1.42	1.32	8	10	6	11
49	20	F	1.58	53	21.23	75	99	0.76	118	78	72	67	30.6	69.4	0.44	1.16	-8	-4	1.25	1.68	4	8	8	15
50	21	M	1.62	56	21.34	74	99	0.75	110	70	74	56	46.8	53.2	0.88	1.18	-4	0	1.24	1.54	6	12	10	5

MASTER CHART CASES

S.NO	AGE (YRS)	SEX (M/F)	Ht (m)	Wt (Kg)	BMI (kg/m^2)	WAIST (cms)	HIP cms)	WHR	SBP (mm Hg)	DBP (mm Hg)	Mean HR (/min)	SDNN (msec)	LF nu	HF nu	LF/HF	OST 30/15 ratio	OST SBP_1	OST DBP_1	E/I ratio	VR	IHG SBP	IHG DBP	CPT DBP	S.Leptin (ng/ml)
1	22	F	1.58	82	32.85	112	116	0.97	120	70	74	46	48.6	51.4	0.95	1.13	6	16	1.18	1.16	8	4	6	30
2	19	M	1.7	90	31.14	106	106	1.00	104	64	72	42	57.2	42.8	1.34	1.16	8	10	1.21	1.21	4	0	12	16
3	23	M	1.62	89	33.91	114	111	1.03	100	62	75	55	64.3	35.7	1.80	1.08	4	14	1.19	1.23	6	2	8	15
4	20	F	1.54	78	32.89	109	116	0.94	98	60	78	45	52.4	47.6	1.10	1.14	12	14	1.14	1.17	-8	-2	14	31
5	19	F	1.57	81	32.86	110	114	0.96	114	68	76	38	54.7	45.3	1.21	1.08	10	18	1.18	1.16	4	0	10	24
6	20	M	1.63	82	30.86	107	106	1.01	124	72	74	44	56.2	43.8	1.28	1.22	4	14	1.24	1.56	-4	-2	8	17
7	18	F	1.53	76	32.47	113	115	0.98	110	66	74	57	64.3	35.7	1.80	1.12	8	12	1.13	1.14	6	2	12	23
8	18	M	1.61	80	30.86	108	107	1.01	100	64	77	42	57.2	42.8	1.34	1.19	6	14	1.22	1.22	-6	-4	10	20
9	21	M	1.64	80	29.74	104	103	1.01	116	60	75	58	61.2	38.8	1.58	1.14	8	14	1.25	1.38	-4	0	10	19
10	22	M	1.66	79	28.67	100	100	1.00	100	64	74	56	43.6	56.4	0.77	1.21	4	10	1.32	1.42	-2	0	8	18
11	24	F	1.59	78	30.85	107	109	0.98	118	70	73	52	64.2	35.8	1.79	1.18	10	16	1.14	1.12	4	0	16	22
12	22	F	1.65	83	30.49	106	108	0.98	130	72	78	49	52.8	47.2	1.12	1.13	8	12	1.09	1.26	8	2	10	25
13	18	M	1.58	70	28.04	100	101	0.99	124	72	73	52	66.4	33.6	1.98	1.21	12	14	1.33	1.54	-8	-4	4	16
14	20	M	1.72	84	28.39	101	101	1.00	120	74	72	54	32.6	67.3	0.48	1.18	8	12	1.32	1.41	-2	0	10	11
15	19	F	1.52	74	32.03	104	106	0.98	124	72	84	39	52.4	47.6	1.10	1.03	10	18	1.35	1.34	4	4	6	27
16	21	F	1.4	68	34.69	112	115	0.97	128	66	79	52	54.5	45.5	1.20	0.86	8	12	1.12	1.16	12	6	18	32
17	18	F	1.51	73	32.02	108	112	0.96	120	68	75	65	66.7	33.3	2.00	1.13	6	14	1.18	1.31	4	2	14	24
18	19	F	1.59	69	27.29	99	102	0.97	114	74	76	58	59.1	40.9	1.44	1.22	4	10	1.24	1.46	6	0	8	25
19	22	M	1.6	70	27.34	102	103	0.99	110	72	74	54	46.8	54.2	0.86	1.21	6	8	1.33	1.38	-4	-2	6	16
20	23	F	1.54	73	30.78	107	109	0.98	116	70	72	66	53.4	46.6	1.15	1.14	4	12	1.16	1.26	6	0	10	19
21	18	M	1.62	84	32.01	105	104	1.01	124	74	83	43	59.8	40.2	1.49	0.94	8	10	1.1	1.18	4	4	12	23
22	19	M	1.63	86	32.37	106	104	1.02	126	80	76	68	55.8	44.2	1.26	1.15	4	16	1.24	1.24	-8	-4	6	25
23	18	F	1.55	74	30.80	100	102	0.98	120	78	78	37	44.7	55.3	0.81	1.22	8	14	1.24	1.12	6	2	8	29
24	18	F	1.5	78	34.67	113	115	0.98	118	72	86	47	74.5	25.5	2.92	1.14	12	18	1.14	1.19	14	8	16	37
25	24	M	1.63	82	30.86	102	102	1.00	116	74	76	39	55.5	44.5	1.25	1.21	6	10	1.26	1.25	4	0	10	21

MASTER CHART CASES

S.NO	AGE (YRS)	SEX (M/F)	Ht (m)	Wt (Kg)	BMI (kg/m^2)	WAIST (cms)	HIP cms)	WHR	SBP (mm Hg)	DBP (mm Hg)	Mean HR (/min)	SDNN (msec)	LF nu	HF nu	LF/HF	OST 30/15 ratio	OST SBP_1	OST DBP_1	E/I ratio	VR	IHG SBP	IHG DBP	CPT DBP	S.Leptin (ng/ml)
26	20	M	1.53	80	34.17	111	114	0.97	124	72	84	55	69.4	30.6	2.27	0.92	4	14	1.02	1.17	8	4	10	35
27	18	M	1.66	84	30.48	101	101	1.00	114	82	74	69	59.7	40.3	1.48	1.12	6	12	1.23	1.34	4	0	6	17
28	19	F	1.49	70	31.53	104	110	0.95	128	82	82	54	62.3	37.5	1.66	1.21	10	18	1.21	1.14	6	4	12	39
29	22	M	1.56	73	30.00	100	100	1.00	114	76	78	68	50.8	49.2	1.03	1.19	4	12	1.24	1.21	6	2	10	11
30	19	F	1.59	73	28.88	98	100	0.98	114	74	72	64	36.6	63.3	0.58	1.14	8	14	1.42	1.49	-8	-2	8	21
31	23	M	1.67	74	26.53	96	98	0.98	110	70	74	60	28.8	71.2	0.40	1.22	8	22	1.48	1.44	-4	0	14	16
32	18	F	1.6	80	31.25	105	108	0.97	124	86	78	59	58.4	40.6	1.44	1.15	6	14	1.26	1.48	8	4	6	28
33	23	F	1.47	71	32.86	106	107	0.99	122	84	82	57	63.2	36.8	1.72	0.86	10	16	1.17	1.17	10	6	8	35
34	18	F	1.57	73	29.62	104	108	0.96	118	82	72	38	58.8	41.2	1.43	1.22	4	12	1.34	1.42	-6	-4	8	25
35	21	M	1.52	80	34.63	113	113	1.00	124	82	78	46	68.5	31.5	2.17	0.78	6	24	1.16	1.16	14	8	12	33
36	19	M	1.65	82	30.12	108	109	0.99	112	70	74	56	48.6	51.4	0.95	1.18	12	16	1.24	1.31	-4	0	10	13
37	19	F	1.56	81	33.28	107	108	0.99	110	70	72	40	55.5	49.5	1.12	1.21	8	16	1.18	1.21	6	2	8	38
38	24	M	1.45	72	34.24	116	115	1.01	118	72	83	52	59.4	41.5	1.43	0.86	6	12	1.24	1.22	10	4	6	18
39	22	M	1.71	84	28.73	98	98	1.00	122	78	74	57	26.4	75.6	0.35	1.18	4	14	1.36	1.42	-2	0	8	15
40	23	F	1.69	79	27.66	98	102	0.96	112	74	76	52	59.5	40.5	1.47	1.21	8	14	1.32	1.35	-8	-4	8	26
41	23	F	1.53	78	33.32	110	109	1.01	126	72	84	56	64.2	34.7	1.85	0.98	6	8	1.15	1.16	8	4	14	24
42	18	F	1.55	80	33.30	110	110	1.00	118	74	80	48	75.4	24.6	3.07	0.94	12	18	1.23	1.18	6	2	12	28
43	21	M	1.6	84	32.81	104	104	1.00	114	78	76	53	49.8	50.2	0.99	1.18	14	20	1.33	1.3	-4	0	8	19
44	19	M	1.62	87	33.15	109	105	1.04	114	76	82	57	53.2	43.8	1.21	1.14	8	14	1.16	1.42	10	4	12	22
45	18	F	1.56	84	34.52	115	116	0.99	116	74	84	58	64.3	35.7	1.80	1.02	6	14	1.32	1.22	12	6	10	39
46	24	M	1.65	88	32.32	109	107	1.02	110	74	74	45	58.4	41.6	1.40	1.22	8	12	1.28	1.19	-4	-4	16	26
47	25	F	1.54	81	34.15	102	98	1.04	116	78	82	46	66.8	33.2	2.01	0.89	4	18	1.18	1.16	8	4	10	36
48	18	M	1.53	81	34.60	117	120	0.98	122	80	74	51	68.4	31.6	2.16	1.04	6	12	1.24	1.21	14	10	6	29
49	18	F	1.58	86	34.45	116	117	0.99	120	80	86	58	70.4	29.6	2.38	0.98	6	14	1.2	1.23	8	4	6	36
50	22	M	1.59	75	29.67	103	103	1.00	120	84	74	53	34.8	65.2	0.53	1.19	4	10	1.32	1.38	-4	0	8	20